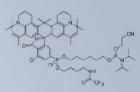
Molecular Diagnostics

0.9

REAGENTS FOR OLIGONUCLEOTIDE LABELING

Product Guide





2023



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1. Introduction

Toolbox for oligonucleotide labeling

ELITechGroup[®] has been actively involved in the development of fluorescence-based nucleic acid probe technologies since the early 1990s. This experience has helped us better understand the challenges of nucleic acid probe manufacturing and the needs of end users. The proprietary reagents and techniques that we have been developing have two main goals in mind: first, to provide labeled probes with the desired physicochemical properties, and, second, to simplify the probes' synthesis and purification. To achieve these goals, we have put together a comprehensive toolbox for nucleic acid labeling which includes a wide range of unique reagents:

- Fluorescent dyes for signal detection
- Quenchers for signal control
- Nucleic Acid Base Analogues (Super Bases) for modulation of nucleic acid duplex stability
- Universal Nucleic Acid Bases to control hybridization specificity
- **Minor Groove Binders (MGBs)** to enhance DNA duplex stability and hybridization specificity
- Intercalators for modulation of nucleic acid duplex stability
- Artificial Nucleic Acids for orthogonal nucleic acid technologies
- Lipophilic groups for therapeutic nucleic acid applications
- **Spacers and Linkers** to assemble various nucleic acid probe components together and optimize their performance.

These are offered in a variety of reagent forms:

- 2-Cyanoethyl Phosphoramidites for internal and 5'-end labeling
- Synthesis solid supports for 3'-end labeling
- Activated esters for post-synthesis conjugation with aminecontaining substrates

In addition, all of our Super Bases are available as both 3'- and 5'phosphoramidites to make them compatible with both standard $(3'\rightarrow 5')$ and 'reverse' $(5'\rightarrow 3')$ DNA synthesis approaches. The reverse approach offers additional synthesis flexibility, for instance, in situations when a synthesis solid support is only available for 5'-end labeling.

Toolbox applications: DNA probe-based detection chemistries

Numerous DNA detection technologies that rely on fluorophore-labeled nucleic acids have been developed in the last several decades. Simple endlabeled fluorescent probes are useful in heterogeneous assays wherein the target-probe duplex is separated from the excess probe before signal detection (e.g., nucleic acid microarray technologies). On the other hand, most of the nucleic acid amplification technologies (e.g., real-time PCR) are performed in a homogeneous format with no separation of components and require a fluorescence change during or after amplification for target detection. The table below contains examples of some DNA detection chemistries, with the emphasis on ELITechGroup's proprietary MGB technology. More detailed analysis of such chemistries can be found in References 1 and 2. Signal generation for all of these chemistries can generally be attributed to either hydrolysis or hybridization events. The hydrolysis type probes, when annealed to a target, are enzymatically degraded to separate the fluorophore ('Fluor') from a quenching ('Q') moiety, whereas hybridization probes/primers generate fluorescence signal via a hybridization-triggered conformational change. Hybridization probes and primers have the benefit of post-amplification melting curve analysis to confirm the specificity of amplification or detect target mutations.

| Detection Chemistry | Туре | 5′ | 3' | Internal |
|---------------------------|-----------------------------|------------|------------|------------------|
| MGB TaqMan | Hydrolysis probe | Fluor | Q-MGB | N/A |
| MGB Pleiades® | Hybridization probe | Fluor-MGB | Q | N/A |
| MGB Eclipse® | Hybridization probe | Q-MGB | Fluor | N/A |
| DSQ TaqMan | Hydrolysis probe | Fluor | DSQ | N/A |
| MGB FRET | Hybridization probe | Fluor-MGB | Q | Fluor |
| MGB Fluorogenic primer | Fluorogenic primer | Fluor-MGB | None | N/A |
| TaqMan* | Hydrolysis probe | Fluor | Q | N/A |
| Molecular beacon | Hybridization probe | Fluor or Q | Q or Fluor | N/A |
| Scorpion | Fluorogenic probe/primer | Fluor | None | Q and blocker |
| Endonuclease IV | Hydrolysis probe | Q | Fluor | N/A |
| LUX primer | Fluorogenic primer | None | None | Fluor |
| FRET probes | Hybridization probe | Fluor | Fluor | N/A |

*TaqMan is a trademark of Roche Molecular Systems, Inc.

Structurally, the degree of complexity varies among the chemistries. In most cases 5'- or 3'- fluorophore and quencher reagents are involved, as well as 5'- or 3'-MGB for MGB probes. In more complicated cases, internal dyes and spacers are required.

ELITechGroup offers a wide range of dye and MGB reagents for postsynthesis and on-line incorporation to meet the challenges of any of those chemistries. Most of our reagents are available as internal phosphoramidites for internal and multiple dye incorporation. We also offer amine and dye-modified nucleoside phosphoramidites that allow for internal modifications with minimal impact on duplex stability.

MGB TaqMan (Section 9, Figure 13A), **DSQ TaqMan** (Section 12), **TaqMan** probes, **Molecular Beacons**, or **FRET** probes can be synthesized using our quencher synthesis supports (Sections 3, 11, and 12), standard 3'-nucleoside phosphoramidites, and concluded with fluorophore phosphoramidites. Alternatively, **Endonuclease IV** probes, which require a 5'-quencher and a 3'-fluorophore with a special linker to increase mismatch discriminating ability (Ref. 3), are prepared starting from a fluorophore synthesis support followed by 3'-nucleoside phosphoramidites and a quencher phosphoramidite at the last step.

For probes with internal fluorophore or quencher (**Scorpions, LUX primers** or **MGB FRET** (Section 9, Figure 13D) we offer fluorophore/quencher internal phosphoramidites with a non-nucleoside linker or nucleoside phosphoramidites with fluorophore/quencher attached to a nucleobase.

To further increase flexibility, reduce the consumption of valuable MGB and fluorophore reagents, and improve product quality in some cases we recommend using 5'-nucleoside phosphoramidites (a.k.a. 'reverse' phosphoramidites) for probe synthesis. For example, MGB Pleiades probes (Section 9, Figure 13C) contain 5'-MGB, 5'-fluorophore, and 3'-quencher moieties. For this configuration, the most economical synthetic path is to start from a 5'-Fluorophore-MGB synthesis support (Section 13), follow with 5'-nucleoside phosphoramidites for a probe sequence and complete with a quencher phosphoramidite. Having the hydrophobic quencher incorporated at the last step of the synthesis provides an efficient handle for reverse phase HPLC purification. To accommodate this approach with the duplex enhancing Super Bases (Section 5) we offer 'reverse' 5'phosphoramidites of the Super Bases as well. Similarly, MGB Eclipse probes (Section 9, Figure 13B) can be synthesized starting from an EDQ-MGB synthesis support (Section 11), followed bv 5'-nucleoside phosphoramidites addition, and a fluorophore phosphoramidite at the last step. MGB Fluorogenic primers (Section 9, Figure 12), which are analogous to MGB Pleiades with an unmodified 3'-hydroxyl end, can also be synthesized using the 'reverse' phosphoramidite approach.

2. Fluorescent Dyes

ELITechGroup's dyes are available in several fluorescent colors ranging from blue to far red. Their excitation and fluorescence emission spectra (Figure 1 and Figure 2) have been optimized to match filter sets for most commercial fluorescence-based nucleic acid diagnostic instruments. With assay multiplexing in mind, the dyes have also been designed to maximize spectral separation and minimize channel cross-talk.

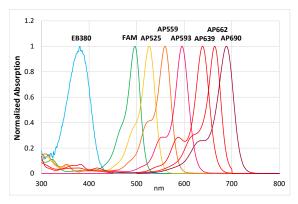


Figure 1 Absorption spectra of representative fluorophores.

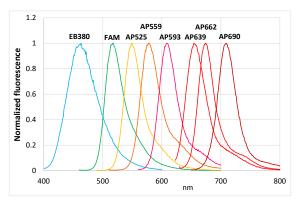


Figure 2 Emission spectra of representative fluorophores.

For most of our fluorophores, we offer three types of labeling reagents: 2cyanoethyl dye phosphoramidites, dye-modified oligonucleotide synthesis supports for on-line dye incorporation and amine-reactive active esters for post-synthesis conjugation. These three types of reagents are detailed below.

2-Cyanoethyl Phosphoramidites

2-Cyanoethyl dye phosphoramidite chemistry is undeniably one of the most convenient methods for the preparation of labeled oligonucleotides. We offer dye phosphoramidites whenever they are synthetically accessible and sufficiently stable during oligonucleotide synthesis and deprotection. Our internal phosphoramidites contain a multifunctional hydroxyprolinol linker with one primary hydroxyl, which is blocked by the DMT group, one secondary hydroxyl for phosphoramidite incorporation, and an amine for dye attachment via an amide bond. This design allows dye incorporation at any desired position within a probe's sequence. In addition, if used in the last coupling cycle, the DMT group offers a convenient DMT-on purification handle, especially for dyes that do not significantly alter C18 HPLC retention.

Synthetically more accessible terminal phosphoramidites with a straight chain C6 linker are only suitable for single dye incorporation at the end of oligonucleotide synthesis but may be a viable alternative to the internal phosphoramidites depending on probe design and purification requirements.

Oligonucleotide Synthesis Supports

Dye-labeled controlled pore glass (CPG) and polystyrene (PS) supports are very economical methods for dye labeling. Depending on whether 5' or 3'-nucleoside phosphoramidites are used, this approach allows essentially quantitative 3' or 5' dye incorporation. All of our dye CPG or PS supports use the proprietary trifunctional hydroxyprolinol moiety, which provides a DMT-blocked primary hydroxyl (the starting point for oligonucleotide synthesis) and a secondary hydroxyl for connecting the dye to the synthesis support via a cleavable succinate linker.

Active esters

Most of our dyes are available as amine-reactive pentafluorophenyl (PFP) esters or fused lactones with activities similar to well-known NHS esters. Both non-aqueous and aqueous/organic conjugation conditions can be used.

Active esters: Post-synthesis conjugation using non-aqueous DMSO conditions

For conjugation applications, one particularly useful property of oligonucleotides is the solubility of their trialkylammonium forms in organic solvents such as DMSO. These forms can be prepared in a variety of ways. For example, a triethylammonium (TEA) form can be obtained by simple C18 reverse phase chromatography (which is usually done prior to a post-synthesis conjugation) using triethylammonium bicarbonate buffer. The volatile buffer can be readily removed by evaporation *in vacuo*. The resultant oligonucleotide (TEA form) is now soluble in DMSO and can be modified with a variety of agents under mostly anhydrous conditions. This approach allows use of conjugation reagents that are either insoluble or unstable in the presence of significant amounts of water, making the conjugation reaction more robust.

Another useful attribute of the non-aqueous protocol is that all oligonucleotide-related material can be easily precipitated by adding a solution of a sodium salt (such as iodide, perchlorate, etc.) in acetone, and separated from the bulk of unreacted dye, thus simplifying conjugate purification.

Overall, our experience indicates that when compared to aqueous/organic conditions, the non-aqueous conjugation procedure is more reproducible; it affords higher coupling yields and simplifies conjugate purification.

Active esters: Two-step conjugation procedures

Preparation of dye-labeled nucleic acid probes by post-synthesis conjugation may present significant challenges in terms of conjugation efficiency and separation of the resultant conjugate from unconjugated dye. The presence of unlabeled oligonucleotide will reduce the probe's quality and ultimately its performance. On the other hand, even minor contamination with unconjugated dye may lead to an undesired increase in background signal.

Conjugates can often be separated from the starting oligonucleotide and unconjugated dye by reverse phase HPLC. It is therefore desirable, for purification purposes, to use fairly hydrophobic dyes that provide significant changes in HPLC retention time. Excessive hydrophobicity, however, may lead to undesired dye aggregation and reduction in the probe's performance. To address this dilemma, we developed a two-step conjugation procedure. In the first step, the main conjugation reaction is done using a transiently protected, hydrophobic form of a dye followed by simple reverse phase HPLC purification. In the second step, the conjugate is treated with a deblocking agent releasing the dye in a more hydrophilic form and purified again. Importantly, the dual HPLC purification procedure simplifies the removal of free, unincorporated dye. This approach is illustrated in Figure 3 for a conjugation reaction between the AquaPhluor 525 lactone (M830683) and an amine-modified oligonucleotide. The AquaPhluor 525 dye, in its fully deprotected form, is highly hydrophilic and provides no useful change in the retention time for HPLC purification. On the other hand, its intermediate has the phosphonate group blocked by the trifluoroacetamidobutyl (TFAAB) groups, which provide a very convenient purification handle. The blocking groups are removed at the next step by treatment with ammonium hydroxide generating the highly polar zwitterionic phosphonate.

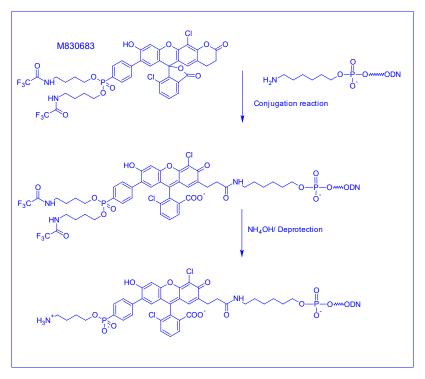


Figure 3 Two-step post-synthesis incorporation of AquaPhluor 525 using its active lactone form (AP525 lactone, M830683).

On-line incorporation of AquaPhluor[®] Dyes

Our proprietary AquaPhluor dyes have been developed for direct on-line incorporation of polar fluorescent dyes into oligonucleotide probes. In their form as labeling reagents, they possess latent phosphonate groups which are fully protected and neutral and, therefore, compatible with the phosphoramidite preparation and coupling chemistries. Upon completion of standard oligonucleotide synthesis, in their ultimate form the dyes are negatively charged and thus significantly more hydrophilic. The phosphonate chemistry of AquaPhluor dyes makes it possible to combine the versatility of automated oligonucleotide synthesis with the benefits of hydrophilicity. As an additional benefit, the phosphonate is a convenient attachment point for a variety of linking and functional groups. Based on how the phosphonate group is utilized, the AquaPhluor dyes can be divided into two classes. AquaPhluor 525, for example, has a phosphonate that is blocked with two TFAAB groups, one of which is eventually removed producing a highly hydrophilic zwitterion (Figure 4).

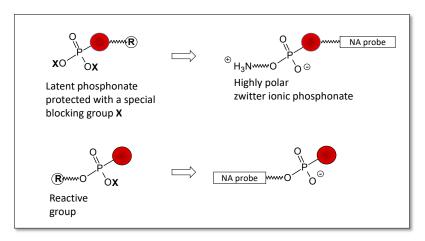
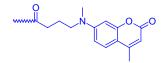


Figure 4 Two types of AquaPhluor chemistries for on-line dye incorporation.

In the second class, which includes AP559, AP593, AP570, AP639, AP642, AP662, AP680 and AP690 dyes, a reactive linking group is integrated as one of the phosphonate blocking groups. After incorporation of the dye into an oligonucleotide and treatment with deblocking agents, only the special blocking group is removed leaving the conjugation linkage intact. Both types of the dyes are compatible with phosphoramidite, active ester, and oligonucleotide chemistries.

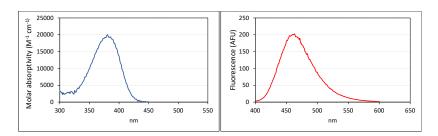
Epoch Blue 380

Acronym: EB380



Epoch Blue 380 is a UV light-excitable blue fluorescent aminocoumarin dye with excitation and emission properties similar to those of Marina Blue and Alexa Fluor 350. It is fully compatible with oligonucleotide synthesis and deprotection.

| Absorbanc | e maximum* | 381 nm |
|------------|--------------------------------|--------------------------------------------------------------|
| Emission m | naximum* | 460 nm |
| Extinction | coefficient (260 nm) | 7,000 M ⁻¹ cm ⁻¹ |
| Extinction | coefficient (260 nm) | 17,200 M ⁻¹ cm ⁻¹ (Epoch Blue 380-HEG) |
| Extinction | coefficient (381 nm) | 20,000 M ⁻¹ cm ⁻¹ |
| Fluorescen | ce quantum yield $^{**}(\Phi)$ | 0.24 |
| Hydrophot | icity by C18 chromatography*** | 22.0 (moderately hydrophobic) |
| pH depend | ence of dye fluorescence | Independent between pH of 5 and 8 |
| | | (Appendix C) |
| | | |



| Available Epoch Blue 380 products | Product number |
|-----------------------------------------|-----------------------------------|
| Epoch Blue 380 PFP ester | M830726 |
| Epoch Blue 380 Internal Phosphoramidite | M830192 |
| Epoch Blue 380-HEG Phosphoramidite | M830727 |
| Epoch Blue 380 CPG | M830669 |
| Epoch Blue 380 Polystyrene**** | M830670 (bulk), M100441 (columns) |

*Measured for T₈-Epoch Blue 380 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

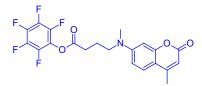
**Measured for T₈-Epoch Blue 380 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-EB380-HEG Dye Calibrator (M300585) from C18 column (see Appendix A for details)

****Suitable for ABI 3900 DNA synthesizer

Epoch Blue 380 PFP ester

Product number: M830726; CAS #: 2378004-31-8

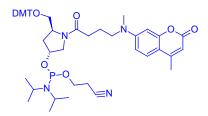


| Formula | C ₂₁ H ₁₆ F ₅ NO ₄ |
|------------------------------------------|----------------------------------------------------------------|
| Molecular weight (reagent) | 441.35 Da |
| Molecular weight (incorporated) | 257.28 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830726 | bulk |
| M830726-1MG | 1 mg |
| M830726-10MG | 10 mg |

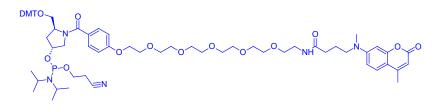
Epoch Blue 380 Internal Phosphoramidite Product number: M830192; CAS #: 2378004-42-1



| Formula | $C_{50}H_{61}N_4O_8P$ |
|-----------------------------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 877.03 Da |
| Molecular weight (incorporated) | 436.39 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability Available documents | ≤ -10°C, dry/ 8 years Certificate of analysis (CoA) |

| Catalog number | Unit size |
|-----------------|-----------|
| M830192 | bulk |
| M830192-50UMOL | 50 µmol |
| M830192-100UMOL | 100 µmol |
| M830192-250MG | 250 mg |
| M830192-1000MG | 1 g |

Epoch Blue 380-HEG Phosphoramidite Product number: M830727; CAS #: 2378004-26-1



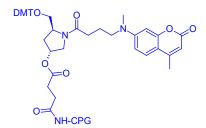
| Formula | $C_{69}H_{90}N_5O_{15}P$ |
|-------------------------------------------|--------------------------------------------------------|
| Molecular weight (reagent) | 1260.45 Da |
| Molecular weight (incorporated) | 819.83 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and |
| | extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ TBD |
| | |

Available documents

| Catalog number | Unit size |
|-----------------|-----------|
| M830727 | bulk |
| M830727-50UMOL | 50 µmol |
| M830727-100UMOL | 100 µmol |
| M830727-250MG | 250 mg |
| M830727-1000MG | 1 g |

Certificate of analysis

Epoch Blue 380 CPG Product number: M830669

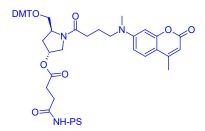


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 436.39 Da |
| Capacity by DMT loading | ≥15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830669 | bulk |
| M830669-100MG | 100 mg |
| M830669-250MG | 250 mg |
| M830669-1000MG | 1 g |

Epoch Blue 380 Polystyrene

Product numbers: M830670 (bulk), M100441 (columns)



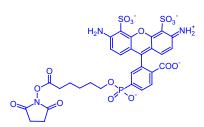
| Formula | N/A |
|-------------------------------------------|---------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 436.39 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or |
| | 2 h at 70°C |
| Recommended storage | 2-8°C, dry/ 4 years |
| conditions/Stability | |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|----------------|----------------------|
| M830670 | bulk |
| M100441-P3 | Pack of 3 columns* |
| M100441-P10 | Pack of 10 columns* |
| M100441-P30 | Pack of 30 columns* |
| M100441-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

AquaPhluor[®] 492 NHS ester

Product number: M830194; CAS #:2378004-15-8

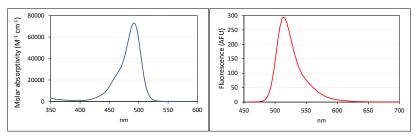


AquaPhluor 492 is a highly hydrophilic fluorescent dye with excitation and emission properties similar to Alexa Fluor 488 and fluorescein. Compared to fluorescein, AP492 is less pH-sensitive and more photostable.

AP492 NHS ester is suitable for conjugation reactions with amine-containing substrates.

| Absorbance maximum |
|-----------------------------------------|
| Emission maximum* |
| Extinction coefficient (260 nm) |
| Extinction coefficient (492 nm) |
| Fluorescence quantum yield**(Φ) |
| Hydrophobicity by C18 chromatography*** |
| pH dependence of dye fluorescence |

492 nm 513 nm 13,500 M⁻¹cm⁻¹ 73,000 M⁻¹cm⁻¹ TBD TBD (hydrophilic) TBD



| Formula | C ₃₀ H ₂₅ Li ₃ N ₃ O ₁₆ PS ₂ (Li salt) |
|--------------------------------------|----------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 799.46 Da |
| Molecular weight (conjugated) | 666.57 Da |
| Purity**** (reverse phase HPLC) | <u>></u> 70% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions: | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stabi | lity \leq -10°C, dry/ 3 years |
| Available documents | CoA, conjugation protocols |

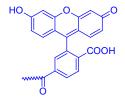
*Measured for T₈-AP492 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP492 conjugate (0.1 M Sodium tetraborate)

Percent acetonitrile required for elution of T_8 -AP492 from C18 column (Appendix A) *Purity of this active derivative is verified by HPLC & NMR and usually exceeds 70%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830194 | bulk |
| M830194-1MG | 1 mg |
| M830194-10MG | 10 mg |

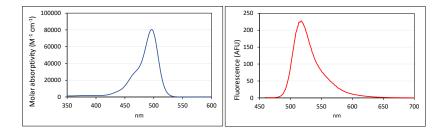
6-FAM



FAM (6-Carboxyfluorescein) is a popular green fluorescent dye compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum* Emission maximum* Extinction coefficient (260 nm) Extinction coefficient (496 nm) Fluorescence quantum yield**(Φ) Hydrophobicity by C18 chromatography*** pH dependence of dye fluorescence 496 nm 517 nm 33,300 M⁻¹cm⁻¹ (FAM-HEG) 80,400 M⁻¹cm⁻¹ 0.88 19.2 (hydrophilic)

pKa 6.8-7.0 (see Appendix C)



| Available FAM products | Product number |
|--------------------------------|-----------------------------------|
| 6-FAM Phosphoramidite | M100011 |
| FAM-HEG Phosphoramidite | M830100 |
| FAM-HEG CPG | M830719 |
| FAM-HEG Polystyrene**** | M830718 (bulk), M100442 (columns) |
| FAM-dU 3'-Phosphoramidite | M830763 |
| FAM-dU 5'-Phosphoramidite | M830764 |
| FAM-Super I 3'-Phosphoramidite | M830783 |

*Measured for T₈-FAM-HEG conjugate (50 mM Tris-HCl pH 8.5, 20°C)

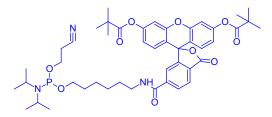
**Measured for T₈-FAM-HEG conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-FAM-HEG Dye Calibrator (M300586)

from C18 column (see Appendix A for details)

****Suitable for ABI 3900 DNA synthesizer

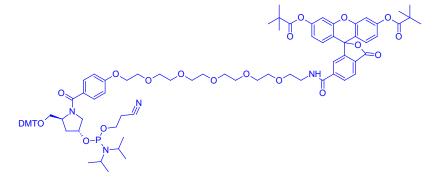
6-FAM Phosphoramidite Product number: M100011; CAS #: 204697-37-0



| Formula | C46H58N3O10P |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 843.94 Da |
| Molecular weight (incorporated) | 537.45 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 10 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M100011 | bulk |
| M100011-50UMOL | 50 µmol |
| M100011-100UMOL | 100 µmol |
| M100011-250MG | 250 mg |
| M100011-1000MG | 1 g |

FAM-HEG Phosphoramidite Product number: M830100; CAS #: 2378004-27-2

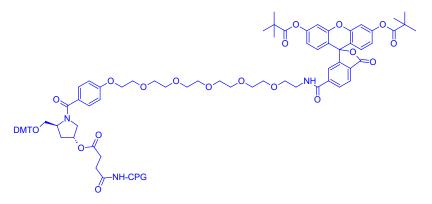


| Formula | C ₈₅ H ₁₀₁ N ₄ O ₂₀ P |
|-------------------------------------------|-------------------------------------------------------------------|
| Molecular weight (reagent) | 1529.70 Da |
| Molecular weight (incorporated) | 920.84 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 10 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830100 | bulk |
| M830100-50UMOL | 50 µmol |
| M830100-100UMOL | 100 µmol |
| M830100-250MG | 250 mg |
| M830100-1000MG | 1 g |

FAM-HEG CPG

Product number: M830719

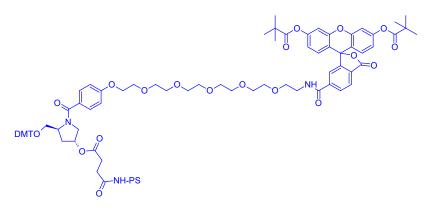


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 920.84 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830719 | bulk |
| M830719-100MG | 100 mg |
| M830719-250MG | 250 mg |
| M830719-1000MG | 1 g |

FAM-HEG Polystyrene

Product numbers: M830718 (bulk), M100256 (columns)

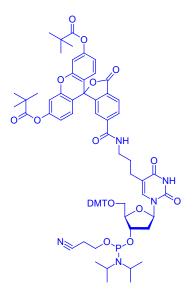


| N/A |
|------------------------------------------|
| N/A |
| 920.84 Da |
| <u>></u> 10 μmol/g |
| <u>></u> 85% |
| N/A |
| 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| or 2 h at 70°C |
| 2-8°C, dry/ 4 years |
| Certificate of analysis |
| |

| Catalog number | Unit size |
|----------------|----------------------|
| M830718 | bulk |
| M100442-P3 | Pack of 3 columns* |
| M100442-P10 | Pack of 10 columns* |
| M100442-P30 | Pack of 30 columns* |
| M100442-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

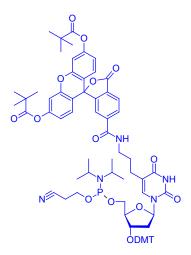
FAM-dU 3'-Phosphoramidite Product numbers: M830763; CAS #: 2378004-02-3



| Formula | $C_{73}H_{80}N_5O_{16}P$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1314.41 Da |
| Molecular weight (incorporated) | 705.56 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 9 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830763 | bulk |
| M830763-50UMOL | 50 µmol |
| M830763-100UMOL | 100 µmol |
| M830763-250MG | 250 mg |
| M830763-1000MG | 1 g |

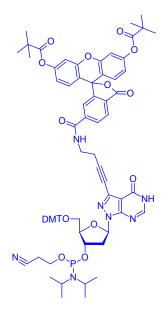
FAM-dU 5'-Phosphoramidite Product numbers: M830764; CAS #: 2378004-07-8



| Formula | C ₇₃ H ₈₀ N ₅ O ₁₆ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 1314.41 Da |
| Molecular weight (incorporated) | 705.56 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 9 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| | |
| M830764 | bulk |
| M830764-50UMOL | 50 µmol |
| M830764-100UMOL | 100 µmol |
| M830764-250MG | 250 mg |
| M830764-1000MG | 1 g |

FAM-Super I 3'-Phosphoramidite Product numbers: M830783; CAS #: 2378004-06-7

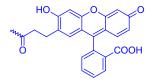


| Formula | C75H78N7O15P |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1348.43 Da |
| Molecular weight (incorporated) | 739.58 Da |
| Purity (reverse phase HPLC) | <u>></u> 85% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 9 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830783 | bulk |
| M830783-50UMOL | 50 µmol |
| M830783-100UMOL | 100 µmol |
| M830783-250MG | 250 mg |
| M830783-1000MG | 1 g |

Gig Harbor Green[™]

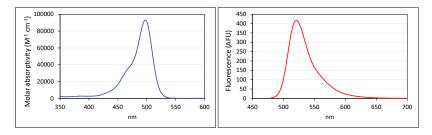
Acronym: GG



Gig Harbor Green is an alternative to FAM with similar excitation and emission properties.

Gig Harbor Green reagents are compatible with standard DNA synthesis and deprotection conditions.

| Absorbance maximum* | 497 nm |
|------------------------------------------|------------------------------------------|
| Emission maximum* | 521 nm |
| Extinction coefficient (260 nm) | 18,500 M ⁻¹ cm ⁻¹ |
| Extinction coefficient (500 nm) | 93,200 M ⁻¹ cm ⁻¹ |
| Fluorescence quantum yield $^{**}(\Phi)$ | 0.85 |
| Hydrophobicity by C18 chromatography*** | 21.5 (hydrophilic) |
| pH dependence of dye fluorescence | pK _a 7.4-7.5 (see Appendix C) |
| | |



| Available Gig Harbor Green products | Product number |
|-------------------------------------|-----------------------------------|
| Gig Harbor Green CPG | M830771 |
| Gig Harbor Green Polystyrene**** | M400090 (bulk), M100433 (columns) |

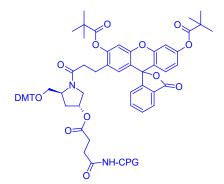
*Measured for T₈-GG conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-GG conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T_8 -GG Dye calibrator from C18 column (see Appendix A for details)

****Suitable for ABI 3900 DNA synthesizer

Gig Harbor Green CPG Product number: M830771

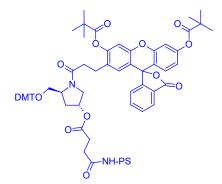


| Formula | N/A |
|-------------------------------------------|-------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 565.46 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830771 | bulk |
| M830771-100MG | 100 mg |
| M830771-250MG | 250 mg |
| M830771-1000MG | 1 g |

Gig Harbor Green Polystyrene

Product numbers: M400090 (bulk), M100433 (columns)



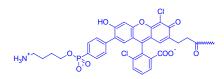
| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 565.46 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 6 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M400090 | bulk |
| M100433-P3 | Pack of 3 columns* |
| M100433-P10 | Pack of 10 columns* |
| M100433-P30 | Pack of 30 columns* |
| M100433-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

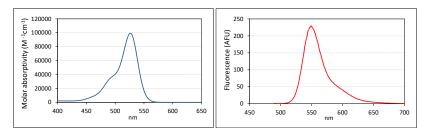
AquaPhluor 525

Acronym: AP525



AquaPhluor 525 is a hydrophilic fluorescent dye with excitation and emission properties similar to VIC, JOE, HEX and Alexa Fluor 532. It possesses a zwitterionic 4-aminobutyl- phosphonate group for increased hydrophilicity and reduced aggregation. The latent phosphonate is conveniently protected in the reactive forms of the dye.

| Absorbance maximum* | 527 nm |
|-----------------------------------------|-----------------------------------------|
| Emission maximum* | 549 nm |
| Extinction coefficient (260 nm) | 41,000 M ⁻¹ cm ⁻¹ |
| Extinction coefficient (527 nm) | 99,300 M ⁻¹ cm ⁻¹ |
| Fluorescence quantum yield**(Φ) | 0.73 |
| Hydrophobicity by C18 chromatography*** | 18.6 (hydrophilic) |
| pH dependence of dye fluorescence | pKa 6.8-7.0 (see Appendix C) |
| | |



| Available AquaPhluor 525 products | Product number |
|-----------------------------------------|-----------------------------------|
| AquaPhluor 525 Lactone | M830683 |
| AquaPhluor 525 Internal Phosphoramidite | M830199 |
| AquaPhluor 525 Terminal Phosphoramidite | M830714 |
| AquaPhluor 525-HEG Phosphoramidite | M100104 |
| AquaPhluor 525 CPG | M830671 |
| AquaPhluor 525 Polystyrene**** | M830672 (bulk), M100276 (columns) |

*Measured for T₈-AP525 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP525 conjugate (0.1 M Sodium tetraborate)

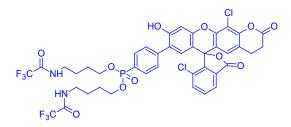
***Percent acetonitrile required for elution of T_8 -AP525 Dye Calibrator (M300587) from

C18 column (see Appendix A for details)

****Suitable for ABI 3900 DNA synthesizer

AquaPhluor 525 Lactone

Product number: M830683; CAS #: 2378004-23-8

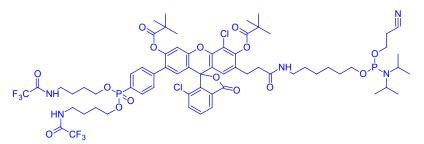


| Formula | $C_{41}H_{33}CI_2F_6N_2O_{11}P$ |
|------------------------------------------|---------------------------------|
| Molecular weight (reagent) | 945.58 Da |
| Molecular weight (incorporated) | 682.44 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Recommended deprotection conditions | See conjugation protocol |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC (via test reaction with an aliphatic amine) & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830683 | bulk |
| M830683-1MG | 1 mg |
| M830683-10MG | 10 mg |

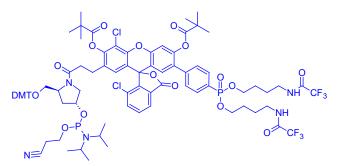
AquaPhluor 525 Terminal Phosphoramidite Product number: M830714; CAS #: 876746-10-0



| Formula | $C_{66}H_{81}CI_2F_6N_5O_{15}P_2$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1431.23 Da |
| Molecular weight (incorporated) | 861.59 Da |
| Purity (reverse phase HPLC) | <u>></u> 85% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830714 | bulk |
| M830714-50UMOL | 50 µmol |
| M830714-100UMOL | 100 µmol |
| M830714-250MG | 250 mg |
| M830714-1000MG | 1 g |

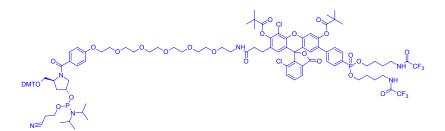
AquaPhluor 525 Internal Phosphoramidite Product number: M830199; CAS #: 2378004-28-3



| Formula | $C_{86}H_{95}Cl_2F_6N_5O_{18}P_2$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1733.54 Da |
| Molecular weight (incorporated) | 861.55 Da |
| Purity (reverse phase HPLC) | <u>></u> 85% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°(or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 9 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830199 | bulk |
| M830199-50UMOL | 50 µmol |
| M830199-100UMOL | 100 µmol |
| M830199-250MG | 250 mg |
| M830199-1000MG | 1 g |

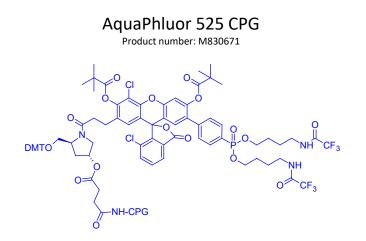
AquaPhluor 525-HEG Phosphoramidite Product number: M100104; CAS #: 2378004-19-2



| Formula | $C_{105}H_{124}Cl_2F_6N_6O_{25}P_2$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 2116.98 Da |
| Molecular weight (incorporated) | 1244.98 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 10 years |
| Available documents | Certificate of analysis |

Certificate of analysis

| Catalog number | Unit size |
|-----------------|-----------|
| M100104 | bulk |
| M100104-50UMOL | 50 µmol |
| M100104-100UMOL | 100 µmol |
| M100104-250MG | 250 mg |
| M100104-1000MG | 1 g |

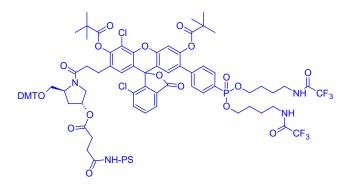


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 861.55 Da |
| Capacity by DMT loading | ≥15 µmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830671 | bulk |
| M830671-100MG | 100 mg |
| M830671-250MG | 250 mg |
| M830671-1000MG | 1 g |

AquaPhluor 525 Polystyrene

Product numbers: M830672 (bulk), M100276 (columns)



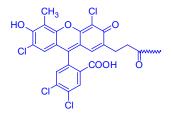
| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 861.55 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°0 |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 8 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830672 | bulk |
| M100276-P3 | Pack of 3 columns* |
| M100276-P10 | Pack of 10 columns* |
| M100276-P30 | Pack of 30 columns* |
| M100276-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

Yakima Yellow®

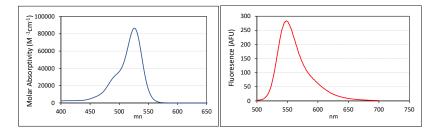
Acronym: YY



Yakima Yellow is a fluorescent dye with excitation and emission properties similar to VIC, JOE, HEX and Alexa Fluor 532. It is fully compatible with standard oligonucleotide synthesis and deprotection.

| Absorbance maximum* |
|-----------------------------------------|
| Emission maximum* |
| Extinction coefficient (260 nm) |
| Extinction coefficient (525 nm) |
| Fluorescence quantum yield**(Φ) |
| Hydrophobicity by C18 chromatography*** |
| pH dependence of dye fluorescence |

525 nm 547 nm 28,600 M⁻¹cm⁻¹ 86,500 M⁻¹cm⁻¹ 0.57 25.6 (moderately hydrophobic) Independent above pH 7 (see Appendix C)



| Available Yakima Yellow products | Product number |
|----------------------------------|-----------------------------------|
| Yakima Yellow Lactone | M830684 |
| Yakima Yellow Phosphoramidite | M830552 |
| Yakima Yellow CPG | M830395 |
| Yakima Yellow Polystyrene**** | M400063 (bulk), M100443 (columns) |

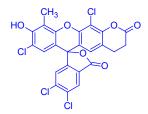
*Measured for T₈-YY conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-YY conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-YY Dye Calibrator (M830730) from C18 column (see Appendix A for details)

Yakima Yellow Lactone

Product number: M830684; CAS #: 502484-58-4

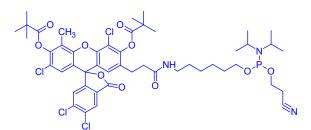


| Formula | $C_{24}H_{12}CI_4O_6$ |
|------------------------------------------|----------------------------|
| Molecular weight (reagent) | 538.16 Da |
| Molecular weight (incorporated) | 538.16 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | < -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC (via test reaction with an aliphatic amine) & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830684 | bulk |
| M830684-1MG | 1 mg |
| M830684-10MG | 10 mg |

Yakima Yellow Phosphoramidite Product number: M830552; CAS #: 502485-39-4



| Formula | C ₄₉ H ₆₀ Cl ₄ N ₃ O ₁₀ P |
|-------------------------------------------|----------------------------------------------------------------------------------|
| Molecular weight (reagent) | 1023.81 Da |
| Molecular weight (incorporated) | 717.31 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830552 | bulk |
| M830552-50UMOL | 50 µmol |
| M830552-100UMOL | 100 µmol |
| M830552-250MG | 250 mg |
| M830552-1000MG | 1 g |

CI

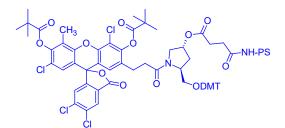
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| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 717.27 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 8 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830395 | bulk |
| M830395-100MG | 100 mg |
| M830395-250MG | 250 mg |
| M830395-1000MG | 1 g |

Yakima Yellow Polystyrene

Product numbers: M400063 (bulk), M100443 (columns)



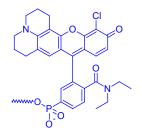
| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 717.27 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M400063 | bulk |
| M100443-P3 | Pack of 3 columns* |
| M100443-P10 | Pack of 10 columns* |
| M100443-P30 | Pack of 30 columns* |
| M100443-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

AquaPhluor 559

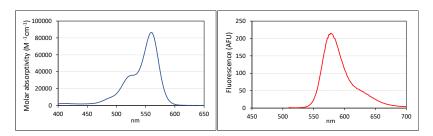
Acronym: AP559



AquaPhluor 559 is a fluorescent dye with excitation and emission properties similar to TAMRA and NED. The dye is compatible with oligonucleotide synthesis and deprotection.

| Absorbance maximum* |
|-----------------------------------------|
| Emission maximum* |
| Extinction coefficient (260 nm) |
| Extinction coefficient (559 nm) |
| Fluorescence quantum yield**(Φ) |
| Hydrophobicity by C18 chromatography*** |
| pH dependence of dye fluorescence |
| |

559 nm 581 nm 28,300 M⁻¹cm⁻¹ 86,500 M⁻¹cm⁻¹ 0.64 25.6 (moderately hydrophobic) Weakly dependent between pH of 5 and 8 (see Appendix C)



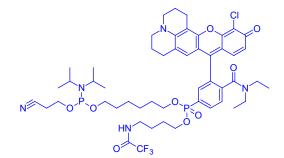
| Available AquaPhluor 559 products | Product number |
|-----------------------------------------|-----------------------------------|
| AquaPhluor 559 Internal Phosphoramidite | M830195 |
| AquaPhluor 559 Terminal Phosphoramidite | M830698 |
| AquaPhluor 559 CPG | M830673 |
| AquaPhluor 559 Polystyrene**** | M830674 (bulk), M100278 (columns) |

*Measured for T₈-AP559 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP559 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP559 Dye Calibrator (M301430) from C18 column (see Appendix A for details)

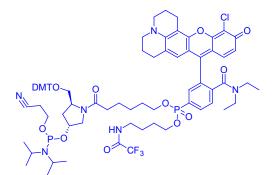
AquaPhluor 559 Terminal Phosphoramidite Product number: M830698; CAS #: 2378004-43-2



| Formula | C51H67CIF3N5O9P2 |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 1048.50 Da |
| Molecular weight (incorporated) | 743.11 Da |
| Purity (reverse phase HPLC) | <u>≥</u> 80% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | \leq -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830698 | bulk |
| M830698-50UMOL | 50 µmol |
| M830698-100UMOL | 100 µmol |
| M830698-250MG | 250 mg |
| M830698-1000MG | 1 g |

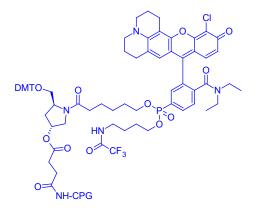
AquaPhluor 559 Internal Phosphoramidite Product number: M830195; CAS #: 2378004-38-5



| Formula | $C_{77}H_{92}CIF_3N_6O_{13}P_2$ |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 1463.98 Da |
| Molecular weight (incorporated) | 856.23 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>≥</u> 50% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830195 | bulk |
| M830195-50UMOL | 50 µmol |
| M830195-100UMOL | 100 µmol |
| M830195-250MG | 250 mg |
| M830195-1000MG | 1 g |

AquaPhluor 559 CPG Product number: M830673

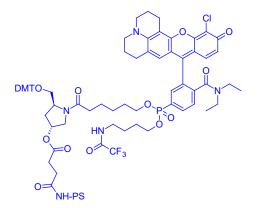


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 856.23 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Coupling efficiency test | N/A |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|----------------|-----------|
| M830673 | bulk |
| M830673-100MG | 100 mg |
| M830673-250MG | 250 mg |
| M830673-1000MG | 1 g |

AquaPhluor 559 Polystyrene

Product numbers: M830674 (bulk), M100278 (columns)



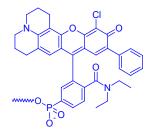
| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 856.23 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830674 | bulk |
| M100278-P3 | Pack of 3 columns* |
| M100278-P10 | Pack of 10 columns* |
| M100278-P30 | Pack of 30 columns* |
| M100278-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

AquaPhluor 570

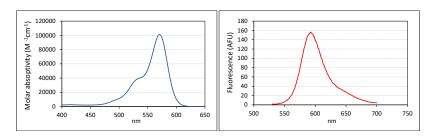
Acronym: AP570



AquaPhluor 570 is a fluorescent dye with excitation and emission properties similar to Redmond Red. Unlike Redmond Red it is pH insensitive and fully compatible with standard oligonucleotide synthesis and deprotection.

Absorbance maximum* Emission maximum* Extinction coefficient (260 nm) Extinction coefficient (570 nm) Fluorescence quantum yield**(Φ) Hydrophobicity by C18 chromatography*** pH dependence of dye fluorescence

| 570 nm |
|------------------------------------------|
| 592 nm |
| 44,100 M ⁻¹ cm ⁻¹ |
| 101,100 M ⁻¹ cm ⁻¹ |
| 0.68 |
| 29.4 (hydrophobic) |
| Weakly dependent between pH 5 and |
| 8 (Appendix C) |



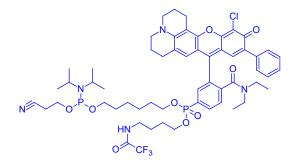
| Available AquaPhluor 570 products | Product number |
|-----------------------------------------|-----------------------------------|
| AquaPhluor 570 Internal Phosphoramidite | M830675 |
| AquaPhluor 570 Terminal Phosphoramidite | M830699 |
| AquaPhluor 570 CPG | M830676 |
| AquaPhluor 570 Polystyrene**** | M830677 (bulk), M100444 (columns) |

*Measured for T₈-AP570 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP570 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP570 Dye Calibrator (M830700) from C18 column (see Appendix A for details)

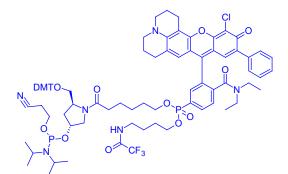
AquaPhluor 570 Terminal Phosphoramidite Product number: M830699; CAS #: 2378004-30-7



| $C_{57}H_{71}CIF_3N_5O_9P_2$ |
|---------------------------------------------------------------|
| 1124.59 Da |
| 819.2 Da |
| <u>></u> 80% |
| <u>></u> 85% |
| ¹ H, ³¹ P NMR /Mass spectroscopy |
| Double phosphoramidite addition and extended coupling time |
| 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| <-10°C, dry/ 2 years |
| Certificate of analysis |
| |

| Catalog number | Unit size |
|-----------------|-----------|
| M830699 | bulk |
| M830699-50UMOL | 50 µmol |
| M830699-100UMOL | 100 µmol |
| M830699-250MG | 250 mg |
| M830699-1000MG | 1 g |

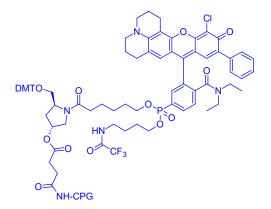
AquaPhluor 570 Internal Phosphoramidite Product number: M830675; CAS #: 2378004-20-5



| Formula | $C_{83}H_{96}CIF_{3}N_{6}O_{13}P_{2}$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1540.08 Da |
| Molecular weight (incorporated) | 932.33 Da |
| Purity (reverse phase HPLC) | <u>></u> 85% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830675 | bulk |
| M830675-50UMOL | 50 µmol |
| M830675-100UMOL | 100 µmol |
| M830675-250MG | 250 mg |
| M830675-1000MG | 1 g |

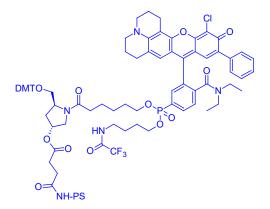
AquaPhluor 570 CPG Product number: M830676



| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 932.33 Da |
| Capacity by DMT loading | ≥15 µmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830676 | bulk |
| M830676-100MG | 100 mg |
| M830676-250MG | 250 mg |
| M830676-1000MG | 1 g |

AquaPhluor 570 Polystyrene Product numbers: M830677 (bulk), M100444 (columns)



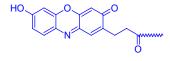
| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 932.33 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830677 | bulk |
| M100444-P3 | Pack of 3 columns* |
| M100444-P10 | Pack of 10 columns* |
| M100444-P30 | Pack of 30 columns* |
| M100444-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

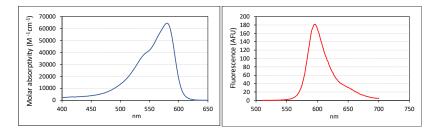
Redmond Red®

Acronym: RR



Redmond Red is a fluorescent dye with excitation and emission properties similar to those of AquaPhluor 570. It is compatible with oligonucleotide synthesis. Mild deprotection conditions are recommended.

580 nm 594 nm 9,800 M⁻¹cm⁻¹ 64,500 M⁻¹cm⁻¹ 0.46 17.9 (hydrophilic) Highly pH-dependent, pK_a ~7 (see Appendix C)



| Available Redmond Red products | Product number |
|--------------------------------|-----------------------------------|
| Redmond Red Lactone | M830728 |
| Redmond Red Phosphoramidite | M830035 |
| Redmond Red CPG | M830390 |
| Redmond Red Polystyrene**** | M830729 (bulk), M100445 (columns) |

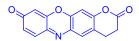
*Measured for T₈-RR conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-RR conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-RR Dye Calibrator (M830731) from C18 column (see Appendix A for details)

Redmond Red Lactone

Product number: M830728; CAS #: 502484-16-4



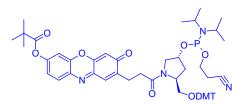
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| Formula | C15H9NO4 |
|------------------------------------------|----------------------------|
| Molecular weight (reagent) | 267.24 Da |
| Molecular weight (incorporated) | 267.24 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC (via test reaction with an aliphatic amine) & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830728 | bulk |
| M830728-1MG | 1 mg |
| M830728-10MG | 10 mg |

Redmond Red Phosphoramidite Product number: M830035; CAS #: 909906-38-3

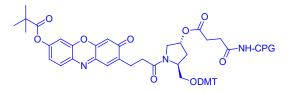


| Formula | C ₅₅ H ₆₃ N ₄ O ₁₀ P |
|-------------------------------------------|---------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 971.08 Da |
| Molecular weight (incorporated) | 446.34 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 10 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830035 | bulk |
| M830035-50UMOL | 50 µmol |
| M830035-100UMOL | 100 µmol |
| M830035-250MG | 250 mg |
| M830035-1000MG | 1 g |
| | |

Redmond Red CPG

Product number: M830390

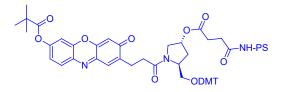


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 446.34 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 3 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830390 | bulk |
| M830390-100MG | 100 mg |
| M830390-250MG | 250 mg |
| M830390-1000MG | 1 g |

Redmond Red Polystyrene

Product numbers: M830729 (bulk), M100445 (columns)



| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 446.34 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Coupling efficiency test | N/A |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 3 years |
| Available documents | Certificate of analysis |
| Available documents | Certificate of analysis |

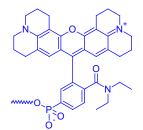
| Catalog number | Unit size |
|----------------|----------------------|
| M830729 | bulk |
| M100445-P3 | Pack of 3 columns* |
| M100445-P10 | Pack of 10 columns* |
| M100445-P30 | Pack of 30 columns* |
| M100445-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

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AquaPhluor 593

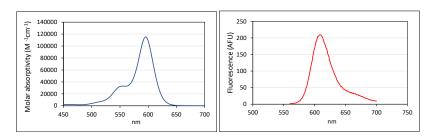
Acronym: AP593



AquaPhluor 593 is a fluorescent dye with excitation and emission properties similar to ROX and Texas Red. The dye is stable under the standard oligonucleotide synthesis and deprotection conditions.

| Absorbance maximum* |
|-----------------------------------------|
| Emission maximum* |
| Extinction coefficient (260 nm) |
| Extinction coefficient (593 nm) |
| Fluorescence quantum yield**(Φ) |
| Hydrophobicity by C18 chromatography*** |
| pH dependence of dye fluorescence |
| |

| 593 nm |
|------------------------------------------|
| 613 nm |
| 19,680 M ⁻¹ cm ⁻¹ |
| 115,240 M ⁻¹ cm ⁻¹ |
| 0.61 |
| 29.9 (hydrophobic) |
| Weakly dependent between pH 5 and 8 |
| (Appendix C) |



| Available AquaPhluor 593 products | Product number |
|-----------------------------------------|-----------------------------------|
| AquaPhluor 593 PFP ester | M830196 |
| AquaPhluor 593 Terminal Phosphoramidite | M830736 |
| AquaPhluor 593 CPG | M830208 |
| AquaPhluor 593 Polystyrene**** | M830732 (bulk), M100257 (columns) |

*Measured for T₈-AP593 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

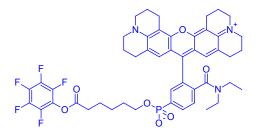
**Measured for T₈-AP593 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP593 Dye Calibrator (M300392) from

C18 column (see Appendix A for details)

AquaPhluor 593 PFP ester

Product number: M830196; CAS #: 1140966-99-9



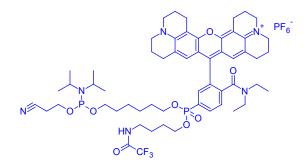
| Formula | C48H49F5N3O7P |
|------------------------------------------|----------------------------|
| Molecular weight (reagent) | 905.88 Da |
| Molecular weight (incorporated) | 721.82 Da |
| Purity* (reverse phase HPLC) @590 nm | <u>></u> 70% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions: | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | < -10°C, dry/ 3 years |
| Available documents | CoA, conjugation protocols |

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*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 70%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830196 | bulk |
| M830196-1MG | 1 mg |
| M830196-10MG | 10 mg |

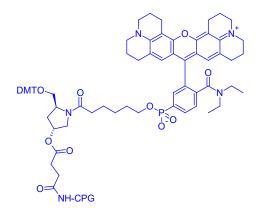
AquaPhluor 593 Terminal Phosphoramidite Product number: M830736; CAS #: 2378004-17-0



| Formula | C57H78F9N6O8P3 |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1239.17 Da |
| Molecular weight (incorporated) | 787.8 Da |
| Purity (reverse phase HPLC) | ≥85% |
| Purity of test oligo (reverse phase HPLC) | ≥80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830736 | bulk |
| M830736-50UMOL | 50 µmol |
| M830736-100UMOL | 100 µmol |
| M830736-250MG | 250 mg |
| M830736-1000MG | 1 g |

AquaPhluor 593 CPG Product number: M830208



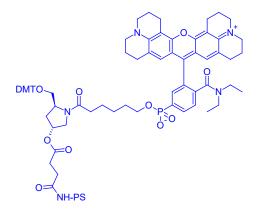
| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 900.93 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830208 | bulk |
| M830208-100MG | 100 mg |
| M830208-250MG | 250 mg |
| M830208-1000MG | 1 g |

_

AquaPhluor 593 Polystyrene

Product numbers: M830732 (bulk), M100257 (columns)



| N/A |
|------------------------------------------------------------|
| N/A |
| 900.93 Da |
| <u>></u> 10 μmol/g |
| <u>></u> 80% |
| N/A |
| 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| 2-8°C, dry/ 7 years |
| Certificate of analysis |
| |

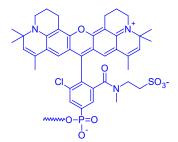
-

| Catalog number | Unit size |
|----------------|----------------------|
| M830732 | bulk |
| M100257-P3 | Pack of 3 columns* |
| M100257-P10 | Pack of 10 columns* |
| M100257-P30 | Pack of 30 columns* |
| M100257-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

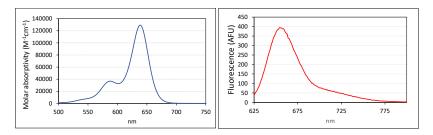
AquaPhluor 639

Acronym: AP639



AquaPhluor 639 is a fluorescent dye with excitation and emission properties similar to Cy5, Quasar 670 and AP642. It features sequence-independent absorption and fluorescence spectra and excellent chemical and photochemical stability. AP639 is compatible with both standard and mild oligonucleotide deprotection conditions.

Absorbance maximum* Emission maximum* Extinction coefficient (260 nm) Extinction coefficient (639 nm) Fluorescence quantum yield**(Φ) Hydrophobicity by C18 chromatography*** pH dependence of dye fluorescence 639 nm 655 nm 24,650 M⁻¹cm⁻¹ 129,300 M⁻¹cm⁻¹ 0.28 37.5 (hydrophobic) Independent between pH 5 and 8 (Appendix C)



| Available AquaPhluor 639 products | Product number |
|-----------------------------------------|---------------------------------------|
| AquaPhluor 639 Terminal Phosphoramidite | M830741 |
| AquaPhluor 639 CPG | M830757 |
| AquaPhluor 639 Polystyrene | M830743 (bulk), M100440 (columns****) |

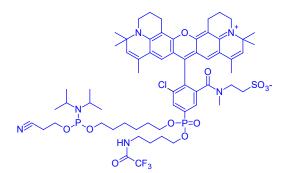
*Measured for T₈-AP639 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP639 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP639 Dye Calibrator (M301715) from

C18 column (see Appendix A for details)

AquaPhluor 639 Terminal Phosphoramidite Product number: M830741; CAS #: 2378724-63-9

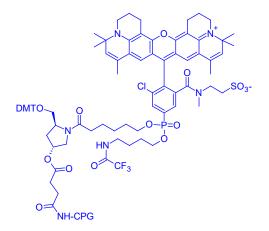


| Formula | $C_{62}H_{82}CIF_{3}N_{6}O_{11}P_{2}S$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1273.81 Da |
| Molecular weight (incorporated) | 968.42 Da |
| Purity (reverse phase HPLC) | ≥80% |
| Purity of test oligo (reverse phase HPLC) | ≥80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | \leq -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830741 | bulk |
| M830741-50UMOL | 50 µmol |
| M830741-100UMOL | 100 µmol |
| M830741-250MG | 250 mg |
| M830741-1000MG | 1 g |

AquaPhluor 639 CPG

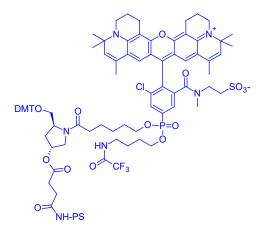
Product numbers: M830757



| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 1081.54 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH4OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 3 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830757 | bulk |
| M830757-100MG | 100 mg |
| M830757-250MG | 250 mg |
| M830757-1000MG | 1 g |

AquaPhluor 639 Polystyrene Product numbers: M830743 (bulk), M100440 (columns)



| N/A |
|------------------------------------------------------------|
| N/A |
| 1081.54 Da |
| >10 µmol/g |
| >70% |
| N/A |
| 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| 2-8°C, dry/ 3 years |
| Certificate of analysis |
| |

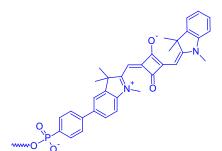
| Catalog number | Unit size |
|----------------|----------------------|
| M830743 | bulk |
| M100440-P3 | Pack of 3 columns* |
| M100440-P10 | Pack of 10 columns* |
| M100440-P30 | Pack of 30 columns* |
| M100440-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

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AquaPhluor 642

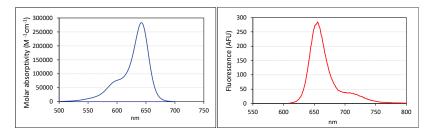
Acronym: AP642



AquaPhluor 642 is a fluorescent dye with excitation and emission properties similar to Cy5. Mild deprotection conditions are recommended for products carrying this dye.

Absorbance maximum* Emission maximum* Extinction coefficient (260 nm) Extinction coefficient (641 nm) Fluorescence quantum yield**(Φ) Hydrophobicity by C18 chromatography*** pH dependence of dye fluorescence

| 641 nm |
|------------------------------------------|
| 653 nm |
| 19,700 M ⁻¹ cm ⁻¹ |
| 283,200 M ⁻¹ cm ⁻¹ |
| 0.13 |
| 33.9 (hydrophobic) |
| Independent between pH 5 and 8 |
| (Appendix C) |
| |



| Available AquaPhluor 642 products | Product number |
|-----------------------------------|-----------------------------------|
| AquaPhluor 642 PFP ester | M830197 |
| AquaPhluor 642 CPG | M830190 |
| AquaPhluor 642 Polystyrene**** | M830678 (bulk), M100279 (columns) |

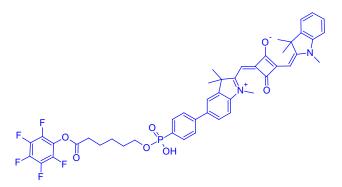
*Measured for T₈-AP642 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP642 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP642 Dye Calibrator (M300589) from C18 column (see Appendix A for details)

AquaPhluor 642 PFP ester

Product number: M830197; CAS #: 2378043-93-5

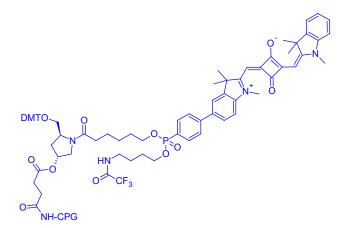


| Formula | C ₄₆ H ₄₂ F ₅ N ₂ O ₇ P (zwitterionic form) |
|------------------------------------------|----------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 860.80 Da |
| Molecular weight (incorporated) | 676.73 Da |
| Purity* (reverse phase HPLC) | <u>></u> 80% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 80%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830197 | bulk |
| M830197-1MG | 1 mg |
| M830197-10MG | 10 mg |

AquaPhluor 642 CPG Product number: M830190

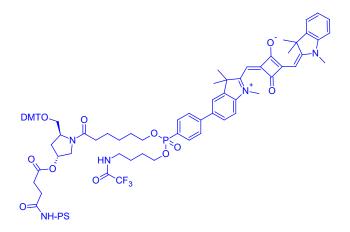


| Formula | N/A |
|-------------------------------------|------------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 855.84 Da |
| Capacity by DMT loading | ≥15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase | <u>></u> 70% |
| HPLC) | |
| Coupling efficiency test | N/A |
| Recommended coupling conditions | N/A |
| Recommended deprotection | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C |
| conditions | or 3 h at 70°C |
| Recommended storage | 2-8°C, dry/ TBD |
| conditions/Stability | |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830190 | bulk |
| M830190-100MG | 100 mg |
| M830190-250MG | 250 mg |
| M830190-1000MG | 1 g |

AquaPhluor 642 Polystyrene

Product numbers: M830678 (bulk), M100280 (200 nmol column), M100279 (1 μmol column)



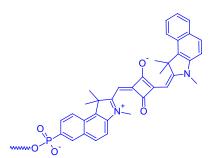
| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 855.84 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 70% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|----------------|----------------------|
| M830678 | bulk |
| M100280-P3 | Pack of 3 columns* |
| M100280-P10 | Pack of 10 columns* |
| M100280-P30 | Pack of 30 columns* |
| M100280-P100 | Pack of 100 columns* |
| M100279-P3 | Pack of 3 columns* |
| M100279-P10 | Pack of 10 columns* |
| M100279-P30 | Pack of 30 columns* |
| M100279-P100 | Pack of 100 columns* |

*ABI 3900 type columns

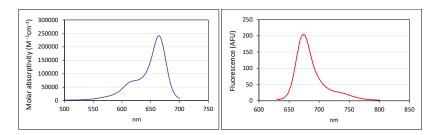
AquaPhluor 662

Acronym: AP662



AquaPhluor 662 is a fluorescent dye with excitation and emission properties similar to those of Cy5. Mild deprotection conditions are recommended for products carrying this dye.

| Absorbance maximum* | 662 nm |
|-----------------------------------------|------------------------------------------|
| Emission maximum* | 672 nm |
| Extinction coefficient (260 nm) | 15,600 M ⁻¹ cm ⁻¹ |
| Extinction coefficient (662 nm) | 241,100 M ⁻¹ cm ⁻¹ |
| Fluorescence quantum yield**(Φ) | 0.14 |
| Hydrophobicity by C18 chromatography*** | 35.0 (hydrophobic) |
| pH dependence of dye fluorescence | Independent between pH 5 and 8 |
| | (Appendix C) |



| Available AquaPhluor 662 products | Product number |
|-----------------------------------|-----------------------------------|
| AquaPhluor 662 PFP ester | M830198 |
| AquaPhluor 662 CPG | M830191 |
| AquaPhluor 662 Polystyrene**** | M830679 (bulk), M100271 (columns) |

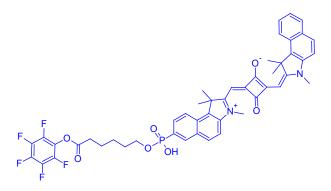
*Measured for T₈-AP662 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP662 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP662 Dye Calibrator (M830724) from C18 column (see Appendix A for details)

AquaPhluor 662 PFP ester

Product number: M830198; CAS #: 2378043-92-4

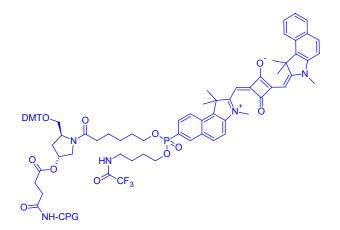


| Formula | C ₄₈ H ₄₂ F ₅ N ₂ O ₇ P (zwitterionic form) |
|------------------------------------------|----------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 884.82 Da |
| Molecular weight (incorporated) | 700.75 Da |
| Purity* (reverse phase HPLC)@640 nm | <u>></u> 85% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | <-10°C, dry/ 5 years |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 85%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830198 | bulk |
| M830198-1MG | 1 mg |
| M830198-10MG | 10 mg |

AquaPhluor 662 CPG Product number: M830191

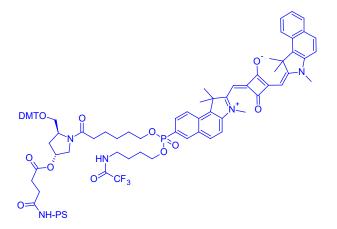


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 879.86 Da |
| Capacity by DMT loading | <u>></u> 15μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830191 | bulk |
| M830191-100MG | 100 mg |
| M830191-250MG | 250 mg |
| M830191-1000MG | 1 g |

AquaPhluor 662 Polystyrene

Product numbers: M830679 (bulk), M100271 (columns)

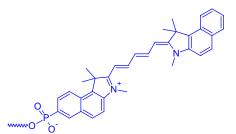


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 879.86 Da |
| Capacity by DMT loading | <u>></u> 8 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 2 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|----------------|----------------------|
| M830679 | bulk |
| M100271-P3 | Pack of 3 columns* |
| M100271-P10 | Pack of 10 columns* |
| M100271-P30 | Pack of 30 columns* |
| M100271-P100 | Pack of 100 columns* |

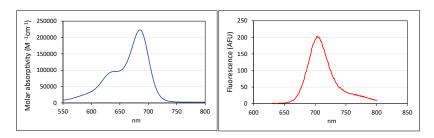
AquaPhluor 680

Acronym: AP680



AquaPhluor 680 is a fluorescent dye with excitation and emission properties similar to those of Cy5.5. Mild deprotection conditions are recommended for products carrying this dye.

Absorbance maximum* Emission maximum* Extinction coefficient (260 nm) Extinction coefficient (682 nm) Fluorescence quantum yield**(Φ) Hydrophobicity by C18 chromatography*** pH dependence of dye fluorescence 682 nm 704 nm 16,100M⁻¹cm⁻¹ 221,300 M⁻¹cm⁻¹ 0.07 35.8** (hydrophobic) Independent between pH 5 & 8 (Appendix C)



| Available AquaPhluor 680 products | Product number |
|-----------------------------------|-----------------------------------|
| AquaPhluor 680 PFP ester | M830682 |
| AquaPhluor 680 CPG | M830680 |
| AquaPhluor 680 Polystyrene**** | M830681 (bulk), M100282 (columns) |

*Measured for T₈-AP680 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

**Measured for T₈-AP680 conjugate (0.1 M Sodium tetraborate)

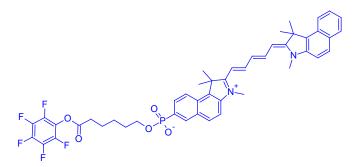
***Percent acetonitrile required for elution of T₈-AP680 Dye Calibrator (M300753) from

C18 column (see Appendix A for details)

****Suitable for ABI 3900 DNA synthesizer

AquaPhluor 680 PFP ester

Product number: M830682; CAS #: 2378004-18-1

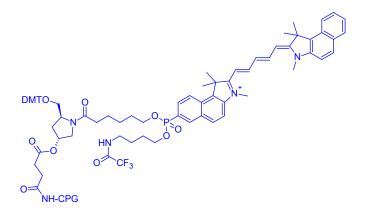


| Formula | C ₄₇ H ₄₄ F ₅ N ₂ O ₅ P (zwitterionic form) |
|------------------------------------------|----------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 842.83 Da |
| Molecular weight (incorporated) | 658.76 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830682 | bulk |
| M830682-1MG | 1 mg |
| M830682-10MG | 10 mg |

AquaPhluor 680 CPG Product number: M830680

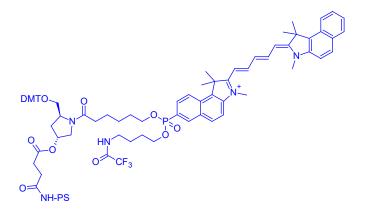


| Formula | N/A |
|-------------------------------------------|----------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 837.88 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1), 8 h at 50°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830680 | bulk |
| M830680-100MG | 100 mg |
| M830680-250MG | 250 mg |
| M830680-1000MG | 1 g |

AquaPhluor 680 Polystyrene

Product numbers: M830681 (bulk), M100282 (columns)

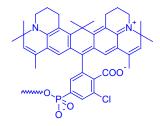


| Formula | N/A |
|-------------------------------------------|---------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 837.88 Da |
| Capacity by DMT loading | <u>≥</u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1), 8 h @ 50°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830681 | bulk |
| M100282-P3 | Pack of 3 columns* |
| M100282-P10 | Pack of 10 columns* |
| M100282-P30 | Pack of 30 columns* |
| M100282-P100 | Pack of 100 columns* |

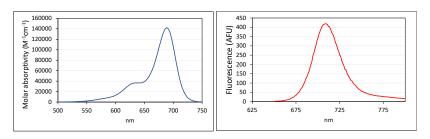
AquaPhluor 690

Acronym: AP690



AquaPhluor 690 is a fluorescent dye with excitation and emission properties similar to AP680, Cy5.5 and Quasar 705. It features sequence-independent absorption and fluorescence spectra and improved chemical and photo-chemical stability as compared to the spectrallysimilar AquaPhluor 680, Cy5.5 and Quasar 705 dyes.

Absorbance maximum* Emission maximum* Extinction coefficient (260 nm) Extinction coefficient (689 nm) Fluorescence quantum yield**(Φ) Hydrophobicity by C18 chromatography*** pH dependence of dye fluorescence 689 nm 709 nm 23,500 M⁻¹cm⁻¹ 141,900 M⁻¹cm⁻¹ 0.14 30.5 (hydrophobic) Independent between pH 5 & 8 (Appendix C)



| Available AquaPhluor 690 products | Product number |
|-----------------------------------|-----------------------------------|
| AquaPhluor 690 Terminal | M830760 |
| Phosphoramidite | |
| AquaPhluor 690 CPG | M830759 |
| AquaPhluor 690 Polystyrene**** | M830755 (bulk), M100500 (columns) |

*Measured for T₈-AP690 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

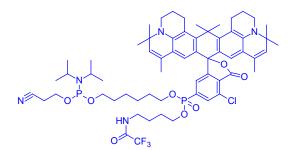
**Measured for T₈-AP690 conjugate (0.1 M Sodium tetraborate)

***Percent acetonitrile required for elution of T₈-AP690 Dye Calibrator (M302123) from

C18 column (see Appendix A for details)

****Suitable for ABI 3900 DNA synthesizer

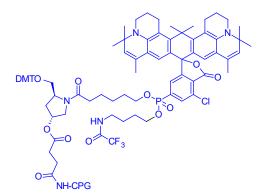
AquaPhluor 690 Terminal Phosphoramidite Product number: M830760; CAS #: 2378004-21-6



| Formula | $C_{62}H_{81}CIF_3N_5O_8P_2$ |
|-------------------------------------------|------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 1178.73 Da |
| Molecular weight (incorporated) | 873.34 Da |
| Purity (reverse phase HPLC) | ≥80% |
| Purity of test oligo (reverse phase HPLC) | ≥80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830760 | bulk |
| M830760-50UMOL | 50 µmol |
| M830760-100UMOL | 100 µmol |
| M830760-250MG | 250 mg |
| M830760-1000MG | 1 g |

AquaPhluor 690 CPG Product numbers: M830759

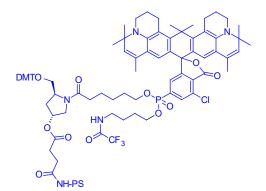


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 986.46 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 70% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830759 | bulk |
| M830759-100MG | 100 mg |
| M830759-250MG | 250 mg |
| M830759-1000MG | 1 g |

AquaPhluor 690 Polystyrene

Product numbers: M830755 (bulk), M100500 (columns)



| N/A |
|-------------------------------------------------------------------------------|
| N/A |
| 986.46 Da |
| <u>></u> 10 μmol/g |
| <u>></u> 65% |
| N/A |
| ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| 55°C or 3 h at 70°C |
| 2-8°C, dry/ 3 years |
| Certificate of analysis |
| |

| Catalog number | Unit size |
|----------------|----------------------|
| M830755 | bulk |
| M100500-P3 | Pack of 3 columns* |
| M100500-P10 | Pack of 10 columns* |
| M100500-P30 | Pack of 30 columns* |
| M100500-P100 | Pack of 100 columns* |

3. Fluorescence Quenchers Azo Dye Quenchers

ELITechGroup's azo dye quenchers have absorption spectra covering fluorophores with emissions from 400 nm to 750 nm (Figure 5). They have been designed with the relatively long range (20-60 Å) fluorescence resonance transfer (FRET) quenching mechanism in mind, which requires a spectral overlap between fluorophore emission and quencher absorption spectra.

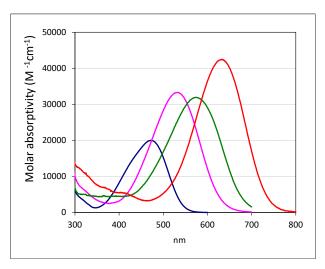


Figure 5 Absorption spectra of azo dye quenchers: Quencher-470 (blue), Eclipse[®] Dark Quencher (pink), Quencher-575 (green), Quencher-630 (red).

Azo dyes are also known to exert non-FRET quenching, so-called contact quenching. Contact quenching (also known as quenching by 'touching' or collisional quenching) is not based on the long range FRET mechanism and requires close contact between fluorophore and quencher. This mechanism works especially well in Molecular Beacons wherein 'touching' is enhanced by the formation of the self-complementary double-stranded stem.

Based on the dynamics of fluorophore-quencher interactions, quenching was categorized as being either dynamic or static in complex formation (Ref. 4). Three different dynamic contact quenching mechanisms were identified: intersystem crossing, electron exchange, and photoinduced electron transfer (PET). At least the last two mechanisms have been shown to be present in known nucleic acid probes. For example, the electron

exchange (also known as Dexter interaction) is present (along with FRET) in linear dual labeled probes (such as TaqMan) and requires temporary orbital overlap. The photoinduced electron transfer between a fluorescent dye and one or more guanine bases, located in close proximity to the dye, is the quenching mechanism for DNA probes described in Ref. 5 and 6. Examples of PET-mediated quenching by nitroindole nucleosides are also described (Ref. 7). It has been shown (Ref. 8) that the CDPI₃-type MGB demonstrates the PET mechanism as well. An example of static quenching is described in Ref. 9; it is characterized by the formation of a ground state complex (hetero-dimer) between a fluorophore and an azo dye quencher accompanied by significant changes in absorption spectra of the dyes.

Contact quenching is present to a varying degree in other probe chemistries that do not have any intentional secondary structure (e.g., TaqMan). For such probes the contribution of contact quenching will generally diminish as the length of the probe increases.

In general, when selecting a quencher, one should consider which quenching mechanism is predominant for a given probe chemistry. For example, for longer non-MGB TaqMan probes, wherein FRET is expected to be the main quenching mechanism, a quencher with the best spectral overlap with fluorophore emission should be selected. On the other hand, for MGB TaqMan or MGB Pleiades probes, which are generally shorter and also benefit from the MGB-induced PET quenching, the Eclipse Dark Quencher will work well regardless of fluorophore emission spectrum.

Lite Quenchers[™]

Lite Quenchers (LQ-380 and LQ-400) are nitrodiarylethene (nitrostilbene) analogues (Ref. 10) whose absorption spectra are substantially blue-shifted relative to the emission spectra of common fluorophores (such as fluorescein). These so-called non-FRET quenchers do not rely on the spectral overlap of quencher absorbance and fluorophore emission (FRET mechanism) for their quenching abilities. They have been designed to eliminate (or significantly weaken) the FRET and preserve the contact quenching. As a result, the oligonucleotide probes possessing such quenchers have fluorescence characteristics that are not always achievable using traditional FRET quenchers.

Lite Quenchers are particularly useful in oligonucleotide hybridization probes having fewer than 12 bases between the fluorophore and quencher. Oligonucleotide probes of this length, when labeled with traditional FRET quenchers, demonstrate significantly reduced fluorescence signals upon

hybridization with their targets. The signal drop is due to insufficient spatial separation between the fluorophore and quencher leading to a residual FRET quenching. The Lite Quenchers, which have substantially diminished FRET quenching properties, avoid this disadvantage.

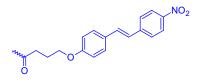
A particular class of probes that is suitable for use in conjunction with the Lite Quenchers are very short (8-12 bases long) MGB Pleiades probes. Such probes are short due to the duplex stabilizing effect of the MGB and are optimal for distinguishing closely related nucleic acid targets. Importantly, these probes containing the Lite Quenchers demonstrate low background fluorescence and high hybridization signals, the latter being significantly higher than those achieved using the traditional FRET-based quenchers. Both background fluorescence and signal are essential parameters for improving assay sensitivity. Such probes are useful, for instance, in digital PCR, such as described in Ref. 11.

Molecular Beacons are another type of probe that may demonstrate improved fluorescence characteristics when used with Lite Quenchers.

Azo dye and Lite Quenchers are available as 2-cyanoethyl dye phosphoramidites, dye-modified oligonucleotide synthesis solid supports for on-line dye incorporation, and amine-reactive esters for post-synthesis conjugation.

Lite Quencher-380

Acronym: LQ-380

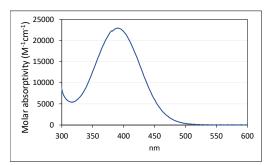


LQ-380 is a contact quencher with no spectral overlap with fluorophores emitting above 500 nm.

Products containing LQ-380 quencher are fully compatible with standard oligonucleotide synthesis and deprotection conditions.

| Absorbance maximum* | 388 nm |
|---------------------------------|-----------------------------------------|
| Extinction coefficient (260 nm) | 9,300 M ⁻¹ cm ⁻¹ |
| Extinction coefficient (388 nm) | 22,900 M ⁻¹ cm ⁻¹ |

*Measured for T₈-LQ-380 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

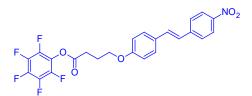


| Available LQ-380 products | Product number |
|--------------------------------------------|-----------------------------------|
| Lite Quencher-380 PFP ester | M830787 |
| Lite Quencher-380 Internal Phosphoramidite | M830786 |
| Lite Quencher-380 CPG | M830799 |
| Lite Quencher-380 Polystyrene** | M830793 (bulk), M100505 (columns) |

**Suitable for ABI 3900 DNA synthesizer

Lite Quencher-380 PFP ester

Product number: M830787; CAS #: 2244400-57-3



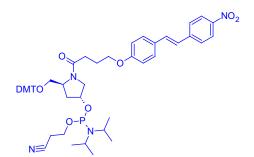
| Formula | C ₂₄ H ₁₆ F ₅ NO ₅ |
|------------------------------------------|----------------------------------------------------------------|
| Molecular weight (reagent) | 493.38 Da |
| Molecular weight (incorporated) | 309.31 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830787 | bulk |
| M830787-1MG | 1 mg |
| M830787-10MG | 10 mg |

Lite Quencher-380 Internal Phosphoramidite

Product number: M830786; CAS #: 2244400-74-4

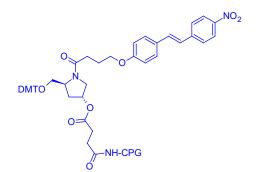


| Formula | C53H61N4O9PP |
|-------------------------------------------|--------------------------------------------------------|
| Molecular weight (reagent) | 929.05 Da |
| Molecular weight (incorporated) | 488.42 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling condition | Double phosphoramidite addition and |
| | extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830786 | bulk |
| M830786-50UMOL | 50 µmol |
| M830786-100UMOL | 100 µmol |
| M830786-250MG | 250 mg |
| M830786-1000MG | 1 g |

Lite Quencher-380 CPG

Product number: M830799

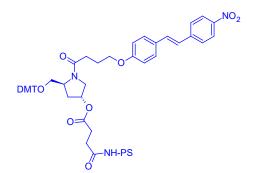


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 488.42 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830799 | bulk |
| M830799-100MG | 100 mg |
| M830799-250MG | 250 mg |
| M830799-1000MG | 1 g |

Lite Quencher-380 Polystyrene

Product numbers: M830793 (bulk), M100505 (columns)

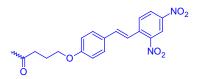


| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 488.42 Da |
| Capacity by DMT loading | ≥10 µmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Coupling efficiency test | N/A |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830793 | bulk |
| M100505-P3 | Pack of 3 columns* |
| M100505-P10 | Pack of 10 columns* |
| M100505-P30 | Pack of 30 columns* |
| M100505-P100 | Pack of 100 columns* |

Lite Quencher-400

Acronym: LQ-400

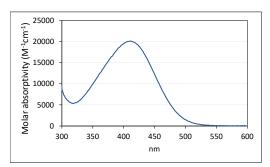


LQ-400 is a contact quencher with a minimal spectral overlap with the emission of fluorescein and no-overlap with fluorophores emitting above 525 nm.

Mild deprotection conditions are recommended for products containing LQ-400 quencher.

| Absorbance maximum* | 407 nm | |
|---------------------------------|-----------------------------------------|--|
| Extinction coefficient (260 nm) | 17,300 M ⁻¹ cm ⁻¹ | |
| Extinction coefficient (407 nm) | 20,000 M ⁻¹ cm ⁻¹ | |

*Measured for T₈-LQ-400 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

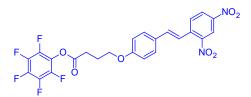


| Available LQ-400 products | Product number |
|-----------------------------------------------|-----------------------------------|
| Lite Quencher-400 PFP ester | M830789 |
| Lite Quencher-400 Internal Phosphoramidite | M830788 |
| Lite Quencher-400 CPG | M830800 |
| Lite Quencher-400 Polystyrene** | M830794 (bulk), M100507 (columns) |

**Suitable for ABI 3900 DNA synthesizer

Lite Quencher-400 PFP ester

Product number: M830789; CAS #: 2244400-58-4



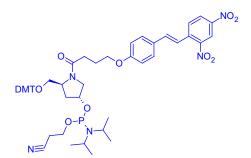
| Formula | C ₂₄ H ₁₅ F ₅ N ₂ O ₇ |
|------------------------------------------|------------------------------------------------------------------------------|
| Molecular weight (reagent) | 538.38 Da |
| Molecular weight (incorporated) | 354.31 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830789 | bulk |
| M830789-1MG | 1 mg |
| M830789-10MG | 10 mg |

Lite Quencher-400 Internal Phosphoramidite

Product number: M830788; CAS #: 2244400-75-5

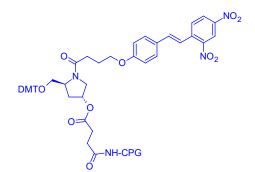


| Formula | C ₅₃ H ₆₀ N ₅ O ₁₁ P |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | 974.04 Da |
| Molecular weight (incorporated) | 533.42 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling condition | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | \leq -10°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| | |
| M830788 | bulk |
| M830788-50UMOL | 50 µmol |
| M830788-100UMOL | 100 µmol |
| M830788-250MG | 250 mg |
| M830788-1000MG | 1 g |

Lite Quencher-400 CPG

Product number: M830800

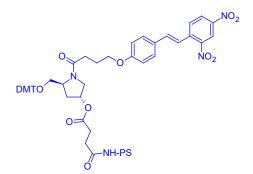


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 533.42 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830800 | bulk |
| M830800-100MG | 100 mg |
| M830800-250MG | 250 mg |
| M830800-1000MG | 1 g |

Lite Quencher-400 Polystyrene

Product number: M830794 (bulk), M100507 (columns)

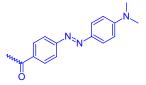


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 533.42 Da |
| Capacity by DMT loading | ≥15 µmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830794 | bulk |
| M100507-P3 | Pack of 3 columns* |
| M100507-P10 | Pack of 10 columns* |
| M100507-P30 | Pack of 30 columns* |
| M100507-P100 | Pack of 100 columns* |

Quencher-470

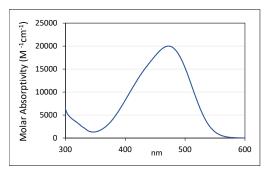
Acronym: Q-470



Quencher-470 is a Dabcyl-based nonfluorescent quencher. Its principal application is a quencher for blue and green fluorescent donor dyes for FRET probes. The quencher is fully compatible with standard oligonucleotide synthesis and deprotection.

| Absorbance maximum* | 470 nm | |
|---------------------------------|-----------------------------------------|--|
| Extinction coefficient (260 nm) | 9,150 M ⁻¹ cm ⁻¹ | |
| Extinction coefficient (470 nm) | 22,800 M ⁻¹ cm ⁻¹ | |

*Measured for T₈-Q470 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

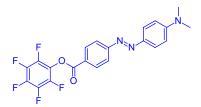


| Available Q470 products | Product number |
|---------------------------------------|-----------------------------------|
| Quencher-470 PFP ester | M830572 |
| Quencher-470 Internal Phosphoramidite | M830565 |
| Quencher-470 CPG | M830562 |
| Quencher-470 Polystyrene** | M830568 (bulk), M100446 (columns) |

**Suitable for ABI 3900 DNA synthesizer

Quencher-470 PFP ester

Product number: M830572; CAS #: 2378004-22-7

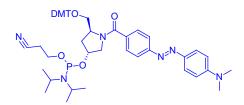


| Formula | $C_{21}H_{14}F_5N_3O_2$ |
|------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | 435.35 Da |
| Molecular weight (incorporated) | 251.28 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 5 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830572 | bulk |
| M830572-1MG | 1 mg |
| M830572-10MG | 10 mg |

Quencher-470 Internal Phosphoramidite Product number: M830565; CAS #: 2378004-32-9

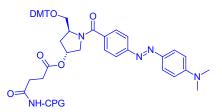


| Formula | $C_{50}H_{59}N_6O_6P$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 871.01 Da |
| Molecular weight (incorporated) | 430.39 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling condition | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| | |
| M830565 | bulk |
| M830565-50UMOL | 50 µmol |
| M830565-100UMOL | 100 µmol |
| M830565-250MG | 250 mg |
| M830565-1000MG | 1 g |

Quencher-470 CPG

Product number: M830562

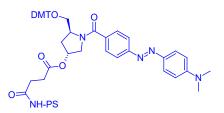


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 430.39 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830562 | bulk |
| M830562-100MG | 100 mg |
| M830562-250MG | 250 mg |
| M830562-1000MG | 1 g |

Quencher-470 Polystyrene

Product numbers: M830568 (bulk), M100446 (columns)

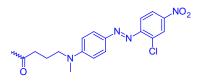


| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 430.39 Da |
| Capacity by DMT loading | ≥10 µmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Coupling efficiency test | N/A |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830568 | bulk |
| M100446-P3 | Pack of 3 columns* |
| M100446-P10 | Pack of 10 columns* |
| M100446-P30 | Pack of 30 columns* |
| M100446-P100 | Pack of 100 columns* |

Eclipse Dark Quencher

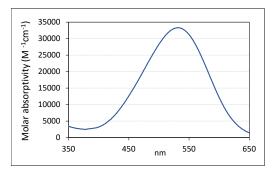
Synonyms: Eclipse Quencher, EDQ



Eclipse dark quencher is a universal nonfluorescent quencher for FRET probes. The quencher is fully compatible with standard oligonucleotide synthesis and deprotection.

| Absorbance maximum* | 530 nm | |
|---------------------------------|-----------------------------------------|--|
| Extinction coefficient (260 nm) | 6,600 M ⁻¹ cm ⁻¹ | |
| Extinction coefficient (530 nm) | 33,300 M ⁻¹ cm ⁻¹ | |

*Measured for T₈-Eclipse Quencher conjugate (50 mM Tris-HCl pH 8.5, 20°C)

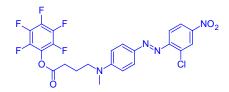


| Available Eclipse Quencher products | Product number |
|---------------------------------------|-----------------------------------|
| Eclipse Dark Quencher PFP ester | M830573 |
| Eclipse Dark Quencher Phosphoramidite | M830028 |
| Eclipse Dark Quencher CPG | M830386 |
| Eclipse Dark Quencher Polystyrene** | M830486 (bulk), M100447 (columns) |
| EDQ-Super I 3'-Phosphoramidite | M830781 |

**Suitable for ABI 3900 DNA synthesizer

Eclipse Dark Quencher PFP ester

Product number: M830573; CAS #: 2378004-34-1

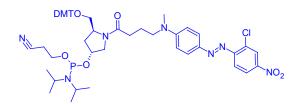


| Formula | C ₂₃ H ₁₆ ClF ₅ N ₄ O ₄ |
|------------------------------------------|--------------------------------------------------------------------------------|
| Molecular weight (reagent) | 542.84 Da |
| Molecular weight (incorporated) | 358.77 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | <-10°C, dry/ 9 years |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830573 | bulk |
| M830573-1MG | 1 mg |
| M830573-10MG | 10 mg |

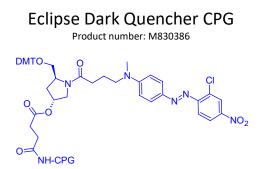
Eclipse Dark Quencher Phosphoramidite Product number: M830028; CAS #: 2378004-24-9



-

| Formula | C ₅₂ H ₆₁ CIN ₇ O ₈ P |
|-------------------------------------------|-------------------------------------------------------------------|
| Molecular weight (reagent) | 978.51 Da |
| Molecular weight (incorporated) | 537.89 Da |
| Purity (reverse phase HPLC) | <u>≥</u> 90% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 10 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830028 | bulk |
| M830028-50UMOL | 50 µmol |
| M830028-100UMOL | 100 µmol |
| M830028-250MG | 250 mg |
| M830028-1000MG | 1 g |

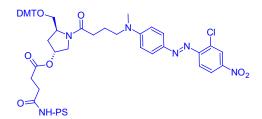


| Formula | N/A |
|-------------------------------------------|-------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 537.89 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830386 | bulk |
| M830386-100MG | 100 mg |
| M830386-250MG | 250 mg |
| M830386-1000MG | 1 g |

Eclipse Dark Quencher Polystyrene

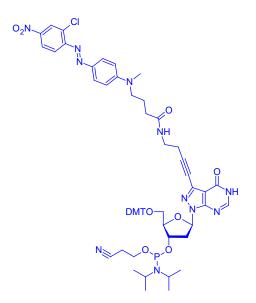
Product numbers: M830486 (bulk), M100447 (columns)



| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 537.89 Da |
| Capacity by DMT loading | ≥10 µmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/10 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830486 | bulk |
| M100447-P3 | Pack of 3 columns* |
| M100447-P10 | Pack of 10 columns* |
| M100447-P30 | Pack of 30 columns* |
| M100447-P100 | Pack of 100 columns* |

EDQ-Super I 3'-Phosphoramidite Product number: M830781; CAS #: 2378004-12-5



| Formula | C ₆₁ H ₆₇ ClN ₁₁ O ₁₀ P |
|-------------------------------------------|---------------------------------------------------------------------|
| Molecular weight (reagent) | 1180.68 Da |
| Molecular weight (incorporated) | 740.05 Da |
| Purity (reverse phase HPLC) | <u>></u> 85% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 3 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830781 | bulk |
| M830781-50UMOL | 50 µmol |
| M830781-100UMOL | 100 µmol |
| M830781-250MG | 250 mg |
| M830781-1000MG | 1 g |

-

Quencher-575

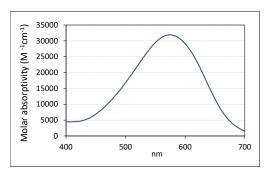
Acronym: Q-575

 NO_2 H₃CO С OCH₃

Quencher-575 quencher is a universal nonfluorescent quencher for FRET probes. The quencher is compatible with standard oligonucleotide synthesis. Mild deprotection is recommended.

| Absorbance maximum* | 575 nm | |
|---------------------------------|-----------------------------------------|--|
| Extinction coefficient (260 nm) | 4,200 M ⁻¹ cm ⁻¹ | |
| Extinction coefficient (530 nm) | 31,900 M ⁻¹ cm ⁻¹ | |

*Measured for T₈-Q575 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

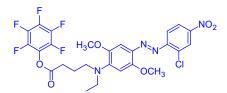


| Available Q575 products | Product number |
|---------------------------------------|-----------------------------------|
| Quencher-575 PFP ester | M830574 |
| Quencher-575 Internal Phosphoramidite | M830566 |
| Quencher-575 CPG | M830563 |
| Quencher-575 Polystyrene** | M830569 (bulk), M100448 (columns) |

**Suitable for ABI 3900 DNA synthesizer

Quencher-575 PFP ester

Product number: M830574; CAS #: 2378004-39-6

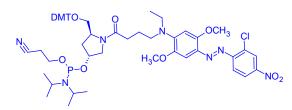


| Formula | C ₂₆ H ₂₂ CIF ₅ N ₄ O ₆ |
|------------------------------------------|--------------------------------------------------------------------------------|
| Molecular weight (reagent) | 616.92 Da |
| Molecular weight (incorporated) | 432.85 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830574 | bulk |
| M830574-1MG | 1 mg |
| M830574-10MG | 10 mg |
| | |

Quencher-575 Internal Phosphoramidite Product number: M830566; CAS #: 2378004-36-3



| Formula | C ₅₅ H ₆₇ CIN ₇ O ₁₀ P |
|-------------------------------------------|---------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 1052.59 Da |
| Molecular weight (incorporated) | 611.96 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 6 years |
| Available documents | Certificate of analysis |

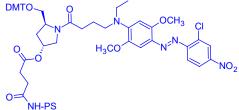
| Catalog number | Unit size |
|-----------------|-----------|
| M830566 | bulk |
| M830566-50UMOL | 50 µmol |
| M830566-100UMOL | 100 µmol |
| M830566-250MG | 250 mg |
| M830566-1000MG | 1 g |

Quencher-575 CPG Product number: M830563 DMTO +N +N+

| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 611.96 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830563 | bulk |
| M830563-100MG | 100 mg |
| M830563-250MG | 250 mg |
| M830563-1000MG | 1 g |

Quencher-575 Polystyrene Product numbers: M830569 (bulk), M100448 (columns)



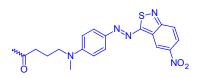
| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 611.96 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 10 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830569 | bulk |
| M100448-P3 | Pack of 3 columns* |
| M100448-P10 | Pack of 10 columns* |
| M100448-P30 | Pack of 30 columns* |
| M100448-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

Quencher-630

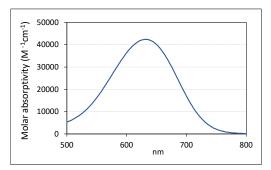
Acronym: Q-630



Quencher-630 is the most red-shifted quencher currently available from ELITechGroup, and it is especially suitable for quenching red and far red fluorophores including the AP680 dye. The quencher is compatible with standard oligonucleotide synthesis. Mild deprotection is recommended.

| Absorbance maximum* | 630 nm | |
|---------------------------------|-----------------------------------------|--|
| Emission maximum* | N/A | |
| Extinction coefficient (260 nm) | 11,160 M ⁻¹ cm ⁻¹ | |
| Extinction coefficient (630 nm) | 42,400 M ⁻¹ cm ⁻¹ | |

*Measured for T₈-Q630 conjugate (50 mM Tris-HCl pH 8.5, 20°C)

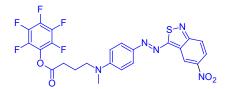


| Available Q630 products | Product number |
|---------------------------------------|-----------------------------------|
| Quencher-630 PFP ester | M830575 |
| Quencher-630 Internal Phosphoramidite | M830567 |
| Quencher-630 CPG | M830564 |
| Quencher-630 Polystyrene** | M830570 (bulk), M100449 (columns) |

**Suitable for ABI 3900 DNA synthesizer

Quencher-630 PFP ester

Product number: M830575; CAS #: 2378004-33-0

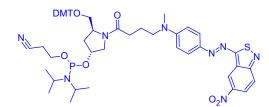


| Formula | $C_{24}H_{16}F_5N_5O_4S$ |
|------------------------------------------|--------------------------------|
| Molecular weight (reagent) | 565.47 Da |
| Molecular weight (incorporated) | 381.40 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830575 | bulk |
| M830575-1MG | 1 mg |
| M830575-10MG | 10 mg |

Quencher-630 Internal Phosphoramidite Product number: M830567; CAS #: 2378004-29-4

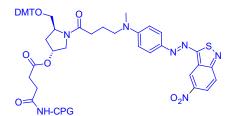


| Formula | $C_{53}H_{61}N_8O_8PS$ |
|-------------------------------------------|---------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 1001.14 Da |
| Molecular weight (incorporated) | 560.51 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | >90% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | \leq -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830567 | bulk |
| M830567-50UMOL | 50 µmol |
| M830567-100UMOL | 100 µmol |
| M830567-250MG | 250 mg |
| M830567-1000MG | 1 g |

Quencher-630 CPG

Product number: M830564

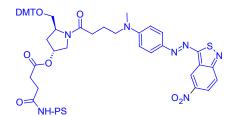


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 560.51 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830564 | bulk |
| M830564-100MG | 100 mg |
| M830564-250MG | 250 mg |
| M830564-1000MG | 1 g |

Quencher-630 Polystyrene

Product numbers: M830570 (bulk), M100449 (columns)

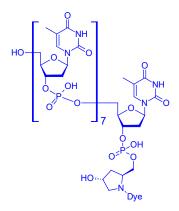


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 560.51 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 3 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830570 | bulk |
| M100449-P3 | Pack of 3 columns* |
| M100449-P10 | Pack of 10 columns* |
| M100449-P30 | Pack of 30 columns* |
| M100449-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

4. Dye Calibrators



Dye calibrators are available for evaluation of our fluorescent dyes' properties and instrument calibrations. The calibrators are T_8 conjugates with the dyes attached to the 3'-terminus via the hydroxyprolinol linker. All conjugates are carefully purified and formulated as 0.1 mM solution in 1x TE buffer.

| Purity by Mass spectroscopy |
|--------------------------------|
| Identity by Mass spectroscopy |
| Recommended storage conditions |
| Stability |
| Available documents |

 \geq 90% ± 0.5% of calculated molecular weight (MW) \leq -10°C, protect from light 3 years at \leq -10°C Certificate of analysis

| Product Number | Dye Calibrator | Abs max (nm) | Em max (nm) | ε ₂₆₀ (M⁻¹cm⁻¹) | ε _{λ max} (M⁻¹cm⁻¹) | MW | Hydro- phobicity* |
|-------------------|---------------------------|--------------------|-------------------|-------------------------------|---------------------------------|--------|----------------------|
| M300585 | T ₈ -EB380-HEG | 381 | 460 | 82,600 | 20,000 | 3191.5 | 22.0 |
| M300586 | T ₈ -FAM-HEG | 496 | 517 | 98,700 | 80,400 | 3292.4 | 19.2 |
| M302132 | T ₈ -GG | 497 | 521 | 18,500 | 93,200 | 3320.6 | 21.5 |
| M300587 | T ₈ -AP525 | 527 | 549 | 117,900 | 99,300 | 3233.2 | 18.6 |
| M830730 | T ₈ -YY | 525 | 547 | 94,000 | 86,500 | 3088.9 | 25.6 |
| M301430 | T ₈ -AP559 | 559 | 581 | 93,700 | 86,500 | 3227.8 | 25.6 |
| M830700 | T ₈ -AP570 | 570 | 592 | 109,500 | 101,100 | 3303.9 | 29.4 |
| M830731 | T ₈ -RR | 580 | 594 | 75,200 | 64,500 | 2817.9 | 17.9 |
| M300392 | T ₈ -AP593 | 593 | 613 | 85,080 | 115,240 | 3272.5 | 29.9 |
| M301715 | T ₈ -AP639 | 639 | 655 | 24,650 | 129,300 | 3453.1 | 37.5 |
| M300589 | T ₈ -AP642 | 641 | 653 | 85,100 | 283,200 | 3227.4 | 33.9 |
| M830724 | T ₈ -AP662 | 662 | 676 | 81,000 | 241,100 | 3251.5 | 35.0 |
| M300753 | T ₈ -AP680 | 682 | 704 | 81,500 | 221,300 | 3209.5 | 35.8 |
| M302123 | T ₈ -AP690 | 689 | 709 | 23,500 | 141,900 | 3358.1 | 30.5 |

All absorption and emission data were obtained in 50 mM Tris-HCl pH 8.5 at 20°C. *Acetonitrile concentration (%) required to elute a dye calibrator from C18 column (see Appendix A for details).

5. Nucleic Acid Base Analogues

In the field of molecular diagnostics, modified nucleobases are often utilized to modulate hybridization properties of natural nucleic acids (NAs). Short NA fragments are used as probes to identify specific NA targets and as primers to amplify these targets. Depending on the application, it may be necessary to adjust duplex stability and specificity. For example, degeneracy of the genetic code, target polymorphism, and a high rate of pathogen mutagenesis often impart strict requirements on assay design, necessitating very short probes and amplification primers. In this case, the hybridization strength of natural NA bases may not be sufficient to satisfy the assay requirements and, therefore, demand the use of some hybridization-enhancing techniques. For other applications, it may be imperative to ignore sequence variations (polymorphisms), which would require overcoming the fundamental specificity of the natural A:T and G:C base-pairs. ELITechGroup has developed a number of artificial NA base analogues to help fulfill those requirements.

Super G[®]

Super G (8-aza-7-deazaguanosine) demonstrates approximately the same base pairing specificity as natural G. However, unlike natural G, due to the absence of the N-7 nitrogen atom, it cannot form non-canonical base pairs. As a result, Super G effectively eliminates undesired secondary structures inherent to natural G-rich sequences and improves hybridization efficiency. The Super G analogue is recommended for use in NA probes and primers to disrupt G stretches.

An additional benefit of Super G for fluorogenic hybridization probes is its low fluorescence quenching tendency, which is an intrinsic and often undesired property of natural G. The resultant Super G-modified probes demonstrate higher fluorescence efficiency and, therefore, assay sensitivity.

Super A[®]

Super A (7-hydroxybutynyl-2-amino-8-aza-7-deazaadenosine) is a duplexstabilizing nucleoside analogue. The Super A:T base pair is much more stable than the natural A:T; it even surpasses the 2-aminoadenosine:T base pair and approaches the stability of the G:C pair. Depending on length and sequence, each Super A incorporation will increase a probe or primer's melting temperature (T_m) by 2.5-5.5°C. Generally, when Super A is located next to a G or C the stabilizing effect is larger (Ref. 14).

Super T[®]

Super T (5-hydroxybutynyl-2'-deoxyuridine) is another duplex-stabilizing nucleoside analogue which forms a stabilized Super T:A base pair with an average T_m increase of about 1.5-3°C per incorporation, presumably due to an increased stacking interaction between nucleotide bases (Ref. 12, 16). The stabilizing effect is highest when several Super Ts are incorporated consecutively.

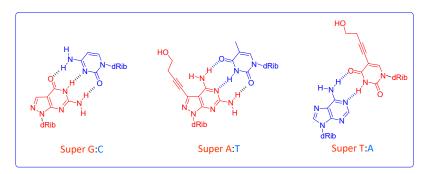


Figure 6 Structures of Super G, Super A, and Super T with their corresponding complementary base pairs.

Guidelines for incorporating Super A and Super T bases into PCR primers

An example of the typical benefits of primers containing modified bases is shown in Figure 7 where they are used to amplify the highly variable *gag* gene region of HIV. Only short sequences are conserved in that region and therefore available for primer design. The unmodified primer pair did not produce any visible PCR products on the agarose gel, whereas the primer pair stabilized by multiple incorporations of Super A and Super T (Forward <u>ACCAAGGAAGC</u>, Reverse CC<u>TICTGATAATGCTG</u>) produced a good band of product with the expected 240 bp size. Other examples of successful Super A and Super T incorporation can be found in the research literature (Ref. 12, 13, 14, 15, 16, 17).

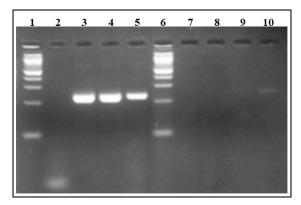


Figure 7 Stabilizing moieties of Super A and Super T in short primers improve amplification of highly variable templates.

Cloned HIV variants (gag gene region) were amplified with 0.5 μ M each of modified or unmodified primers using JumpStart[™] Taq ReadyMix[™] (Sigma, St. Louis, MO, USA) using the following cycling parameters: 2 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. PCR products were run on a 4% agarose gel. Lanes 2-5 indicate the modified primer pair amplification and lanes 7-10 indicated the unmodified primer pair amplification. Lanes 2 & 7 are no template controls. Lanes 3 & 8 contain the pN43 (wild type) plasmid template. Lanes 4 & 9 contain the pN43 – K103N (mutant) plasmid template. Lanes 5 & 10 contain the pN43 – V108I (mutant) plasmid template. Lanes 1 & 6 contain a 100 bp DNA ladder.

It should be noted that PCR efficiency is also affected by factors beyond the thermodynamic properties of primers. DNA polymerase must read through the modified bases without stuttering or pausing, and modified primers have to be efficiently extended. We have conducted experiments and summarized general guidelines for using Super A and Super T in PCR primers:

- Usual PCR T_m requirements should be used to ensure primer annealing.
- Neither Super A nor Super T should be used at the 3' end.
- Both Super A and Super T can be used as the second base from the 3' end.
- Super A should be used sparingly.
- Multiple Super A's should be separated by at least three natural bases or Super T.
- Super T can be used liberally.

Super I[®]

Super I^{TM} is a nucleoside analogue that contains a quasi-universal nucleobase (7-aminobutynyl-8-aza-7-deazahypoxanthine) with improved hybridization and PCR enhancing properties (Ref. 18) as compared to commonly used 2'-deoxyinosine. It demonstrates improved ambiguity for pairing with A, T and C bases and its base pairing properties can be summarized as follows: X:C~X:A~X:T>X:G. The base pairing strength of Super I with A, T and C is approximately equal or slightly greater (depending on nearest neighbors) than the natural A:T but lower than the G:C, with the Super I:C base pair being slightly more stable than Super I:A and Super I:T. The improvement in PCR performance directly correlated with primers' T_m. Primers with multiple Super I incorporations can be used without significant inhibition of Taq polymerase activity provided the modifications are not used in stretches and are positioned more than 2 bases away from the 3' end.

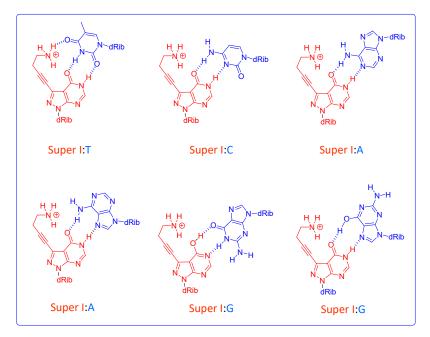
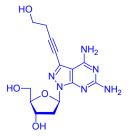


Figure 8 Possible hydrogen bonding patterns of Super I with natural nucleobases.

Comparison of Super I and 2'-deoxyinosine

We have determined that the stabilities of Super I:A, T and C base pairs are approximately equal or slightly greater (depending on nearest neighbors) than the natural A:T but lower than G:C, with Super I:C being slightly more stable than Super I:A and Super I:T. The beneficial stabilization effect of Super I pairs with A, T and C is especially pronounced for the multiplysubstituted duplexes, which are generally more stable than the respective natural matched duplexes. In contrast, 2'-deoxyinosine forms base pairs with all four natural bases that are weaker than the A:T base pair. Moreover, multiple incorporations of 2'-deoxyinosine render modified duplexes much less stable than the respective natural matched duplexes. The much weaker Super I:G base pair is approximately equivalent to dI:G, both of which significantly destabilize the DNA duplexes. For practical applications such as PCR primer design where maintaining primer melting temperature is imperative, these results imply that single and especially multiple substitutions of A, T and G bases with Super I would be thermodynamically favorable whereas substitutions of cytosines should be avoided.

Super A®



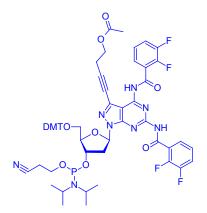
Super A (7-hydroxybutynyl-2-amino-8-aza-7-deazaadenosine) is a duplex-stabilizing nucleoside analogue. The Super A:T base pair is more stable than natural A:T and even surpasses the 2-aminoadenine:T base pair and approaches the stability of the G:C pair. Depending on length and sequence each Super A incorporation will increase the probe or primer's melting temperature (T_m) by 2.5-5.5°C.

Extinction coefficient (260 nm)

8,700 M⁻¹cm⁻¹

| Available Super A products | Product number |
|----------------------------|----------------|
| Super A 3'-Phosphoramidite | M830249 |
| Super A 5'-Phosphoramidite | M830250 |

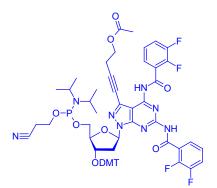
Super A 3'-Phosphoramidite Product number: M830249; CAS #: 2378004-08-9



| Formula | $C_{60}H_{59}F_4N_8O_{10}P$ |
|-------------------------------------------|-------------------------------------------|
| Molecular weight (reagent) | 1159.14 Da |
| Molecular weight (incorporated) | 396.3 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and |
| | extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16h @ 55°C |
| | or 2h @ 70°C. Extra deprotection time may |
| | be needed for multiple incorporations |
| Recommended storage conditions/Stability | < -10°C, dry/ 10 years |
| Available documents | Certificate of analysis, SDS |

| Catalog number | Unit size |
|-----------------|-----------|
| M830249 | bulk |
| M830249-50UMOL | 50 µmol |
| M830249-100UMOL | 100 µmol |
| M830249-250MG | 250 mg |
| M830249-1000MG | 1 g |

Super A 5'-Phosphoramidite Product number: M830250 CAS #: 2378004-04-5



| E a secola | C U ENO B |
|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| Formula | $C_{60}H_{59}F_4N_8O_{10}P$ |
| Molecular weight (reagent) | 1159.14 Da |
| Molecular weight (incorporated) | 396.3 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16h @ 55°C or 2h @ 70°C. Extra time may be needed for multiple incorporations |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 10 years |
| Available documents | Certificate of analysis, SDS |

| Catalog number | Unit size |
|-----------------|-----------|
| M830250 | bulk |
| M830250-50UMOL | 50 µmol |
| M830250-100UMOL | 100 µmol |
| M830250-250MG | 250 mg |
| M830250-1000MG | 1 g |

Super G[®]

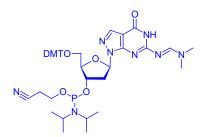
Super G (8-aza-7-deazaguanosine) eliminates undesired secondary structures inherent to natural G-rich sequences and provides improved hybridization efficiency. It is recommended for use in NA probes and primers to disrupt G stretches.

An additional benefit of Super G for use in fluorogenic hybridization probes is its low fluorescence quenching tendency, which is an intrinsic and often undesired property of natural G. The resultant Super G-modified probes demonstrate higher fluorescence efficiency and, therefore, assay sensitivity.

Extinction coefficient (260 nm) 12,400 M⁻¹cm⁻¹

| Available Super G products | Product number |
|----------------------------|----------------|
| Super G 3'-Phosphoramidite | M830006 |
| Super G 5'-Phosphoramidite | M830031 |

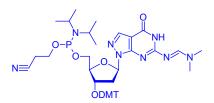
Super G 3'-Phosphoramidite Product number: M830006; CAS #: 500891-26-9



| Formula | C ₄₃ H ₅₃ N ₈ O ₇ P |
|-------------------------------------------|-----------------------------------------------------------------|
| Molecular weight (reagent) | 824.93 Da |
| Molecular weight (incorporated) | 329.21 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 10 years |
| Available documents | Certificate of analysis, SDS |

| Catalog number | Unit size |
|-----------------|-----------|
| M830006 | bulk |
| M830006-50UMOL | 50 µmol |
| M830006-100UMOL | 100 µmol |
| M830006-250MG | 250 mg |
| M830006-1000MG | 1 g |

Super G 5'-Phosphoramidite Product number: M830031; CAS #: 2378004-11-4

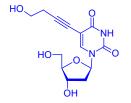


| Formula | C43H53N8O7P |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | 824.93 Da |
| Molecular weight (incorporated) | 329.21 Da |
| Purity (reverse phase HPLC) | >90% |
| Purity of test oligo (reverse phase HPLC) | _ >85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 10 years |
| Available documents | Certificate of analysis, SDS |

_

| Catalog number | Unit size |
|-----------------|-----------|
| M830031 | bulk |
| M830031-50UMOL | 50 µmol |
| M830031-100UMOL | 100 µmol |
| M830031-250MG | 250 mg |
| M830031-1000MG | 1 g |

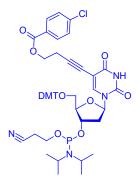
Super T[®]



Super T (5-hydroxybutynyl-2'-deoxyuridine) is another duplex-stabilizing nucleoside analogue which forms a stabilized Super T:A base pair with an average T_m increase of about 2°C per incorporation. The Super T nucleobase can be used liberally in both probe and primer designs with little or no adverse effect on amplification efficiency.

| Available Super T products | Product number |
|----------------------------|----------------|
| Super T 3'-Phosphoramidite | M830040 |
| Super T 5'-Phosphoramidite | M830041 |

Super T 3'-Phosphoramidite Product number: M830040; CAS #: 2378004-13-6



| Formula | C ₅₀ H ₅₄ CIN ₄ O ₁₀ P |
|-------------------------------------------|--------------------------------------------------------------------|
| Molecular weight (reagent) | 937.41 Da |
| Molecular weight (incorporated) | 358.24 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 6 years |
| Available documents | Certificate of analysis, SDS |

| Catalog number | Unit size |
|-----------------|-----------|
| M830040 | bulk |
| M830040-50UMOL | 50 µmol |
| M830040-100UMOL | 100 µmol |
| M830040-250MG | 250 mg |
| M830040-1000MG | 1 g |

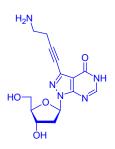
Super T 5'-Phosphoramidite Product number: M830041; CAS #: 2378004-01-2



| Formula | C ₅₀ H ₅₄ CIN ₄ O ₁₀ P |
|-------------------------------------------|--------------------------------------------------------------------|
| Molecular weight (reagent) | 937.41 Da |
| Molecular weight (incorporated) | 358.24 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 6 years |
| Available documents | Certificate of analysis, SDS |

| Catalog number | Unit size |
|-----------------|-----------|
| M830041 | bulk |
| M830041-50UMOL | 50 µmol |
| M830041-100UMOL | 100 µmol |
| M830041-250MG | 250 mg |
| M830041-1000MG | 1 g |

Super I®



Super I is a nucleoside analogue that contains a quasiuniversal nucleobase (7-aminobutynyl-8-aza-7deazahypoxanthine) with improved hybridization and polymerase chain reaction (PCR) enhancing properties as compared to commonly used 2'-deoxyinosine.

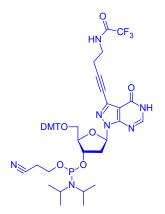
It demonstrates improved ambiguity for pairing with A, T and C bases and its base pairing properties can be summarized as follows: X:C~X:A~X:T>X:G. The base pairing strength of Super I with A, T and C is approximately equal or slightly greater (depending on nearest neighbors) than natural A:T but lower than G:C, with the Super I:C base pair being slightly more stable than Super I:A and Super I:T. The improvement in PCR performance directly correlated with primers' T_m.

Primers with multiple Super I incorporations can be used without noticeable inhibition of Taq polymerase activity provided the modifications are not used in stretches and are positioned more than 2 bases away from the 3' end.

| Extinction coefficient (260 nm) | 10,000 M ⁻¹ cm ⁻¹ |
|---------------------------------|-----------------------------------------|
| | |

| Available Super I products | Product number |
|----------------------------|----------------|
| Super I 3'-Phosphoramidite | M830193 |
| Super I 5'-Phosphoramidite | M830712 |

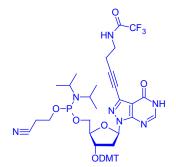
Super I 3'-Phosphoramidite Product number: M830193; CAS #: 1401110-57-3



| Formula | C ₄₆ H ₅₁ F ₃ N ₇ O ₈ P |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 917.91 Da |
| Molecular weight (incorporated) | 381.28 Da |
| Purity (reverse phase HPLC) | <u>≥</u> 90% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | Pre-treatment with 5% N,N,N',N'- tetramethylguanidine in CH ₃ CN followed by 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 8 years |
| Available documents | Certificate of analysis, SDS |

| Catalog number | Unit size |
|-----------------|-----------|
| M830193 | bulk |
| M830193-50UMOL | 50 µmol |
| M830193-100UMOL | 100 µmol |
| M830193-250MG | 250 mg |
| M830193-1000MG | 1 g |

Super I 5'-Phosphoramidite Product number: M830712; CAS #: 2378004-03-4



| Formula | C ₄₆ H ₅₁ F ₃ N ₇ O ₈ P |
|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 917.91 Da |
| Molecular weight (incorporated) | 381.28 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | Pre-treatment with 5% N,N,N',N'- tetramethylguanidine in CH₃CN followed by 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 8 years |
| Available documents | Certificate of analysis, SDS |

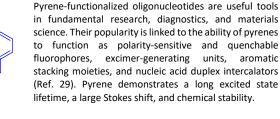
| Catalog number | Unit size |
|-----------------|-----------|
| M830712 | bulk |
| M830712-50UMOL | 50 µmol |
| M830712-100UMOL | 100 µmol |
| M830712-250MG | 250 mg |
| M830712-1000MG | 1 g |

6. Duplex Intercalators Pyrene Internal Phosphoramidite

Product number: M830711; CAS #: 2378724-62-8

HN

DMTO



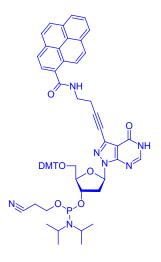
Pyrene internal phosphoramidite (M830711) is prepared by coupling of 1-pyrenecarboxylic acid to D-threoninol, a trifunctional linker with an amino group and two hydroxyl groups in a specific steric bond configuration. The pyrene moiety in this combination is a universal base capable of binding equally well with all four natural nucleobases (unpublished data). To achieve optimal backbone configuration the phosphoramidite should be used in conjunction with 5'-nucleoside phosphoramidites for probe synthesis.

The phosphoramidite is suitable for internal as well as terminal incorporation and compatible with standard DNA synthesis and both standard and mild deprotection conditions.

| Formula | $C_{51}H_{54}N_3O_6P$ |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 835.96 Da |
| Molecular weight (incorporated) | 395.34 Da |
| Extinction coefficient (260 nm) | 12,000 M ⁻¹ cm ⁻¹ |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 7 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|-----------------|-----------|
| M830711 | bulk |
| M830711-50UMOL | 50 µmol |
| M830711-100UMOL | 100 µmol |
| M830711-250MG | 250 mg |
| M830711-1000MG | 1 g |

Pyrene-Super I 3'-Phosphoramidite Product number: M830782; CAS #: 2378004-05-6



| Formula | $C_{61}H_{60}N_7O_8P$ |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 1050.14 Da |
| Molecular weight (incorporated) | 609.52 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830782 | bulk |
| M830782-50UMOL | 50 µmol |
| M830782-100UMOL | 100 µmol |
| M830782-250MG | 250 mg |
| M830782-1000MG | 1 g |

7. 3'-Deoxyribopyranosyl (4' \rightarrow 2') Nucleic Acid

Acronym: p-DNA



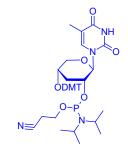
3'-Deoxy- β -D-ribopyranosyl nucleic acids (p-DNA) are polymers that preferentially pair with p-DNA versus natural DNA sequences (Ref. 30). p-DNAs form antiparallel, exclusively Watson-Crick-paired duplexes that are much stronger than corresponding DNA duplexes.

For some applications, it is essential to be able to design large numbers of nucleic acid sequences with the same T_m that hybridize efficiently and specifically with respective targets while not cross-hybridizing with each other (Ref. 31). These so-called T_m-leveled orthogonal nucleic acids can be designed using p-DNA monomers (Ref. 32). Orthogonal p-DNAs with high duplex stability, low affinity for DNA, and which are not recognized by DNA processing enzymes are useful for labeling, barcoding, or anchoring multiple DNA-containing substrates co-existing in one mixture, on one array, or on a lateral flow strip.

p-DNA monomers are available as 4'-DMT, 2'-phosphoramidites and are fully compatible with standard DNA synthesis. In particular, synthesis of chimeric DNA – p-DNA can be achieved via standard synthesis.

| Available p-DNA products | Product number |
|--------------------------|----------------|
| p-DNA T Phosphoramidite | M830779 |
| p-DNA A Phosphoramidite | M830776 |
| p-DNA C Phosphoramidite | M830777 |
| p-DNA G Phosphoramidite | M830778 |

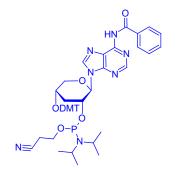
p-DNA T Phosphoramidite Product number: M830779; CAS #: 256648-34-7



| Formula | C40H49N4O8P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 744.81 Da |
| Molecular weight (incorporated) | 304.19 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830779 | bulk |
| M830779-50UMOL | 50 µmol |
| M830779-100UMOL | 100 µmol |
| M830779-250MG | 250 mg |
| M830779-1000MG | 1 g |

p-DNA A Phosphoramidite Product number: M830776; CAS #: 256648-33-6



| Formula | C47H52N7O7P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 857.93 Da |
| Molecular weight (incorporated) | 313.20 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830776 | bulk |
| M830776-50UMOL | 50 µmol |
| M830776-100UMOL | 100 µmol |
| M830776-250MG | 250 mg |
| M830776-1000MG | 1 g |

p-DNA C Phosphoramidite Product number: M830777; CAS #: 2378004-09-0



| Formula | C ₄₆ H ₅₂ N ₅ O ₈ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 833.91 Da |
| Molecular weight (incorporated) | 289.18 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830777 | bulk |
| M830777-50UMOL | 50 µmol |
| M830777-100UMOL | 100 µmol |
| M830777-250MG | 250 mg |
| M830777-1000MG | 1 g |

p-DNA G Phosphoramidite Product number: M830778; CAS #: 457880-65-8



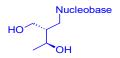
| Formula | C ₄₂ H ₅₀ N ₇ O ₈ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 811.86 Da |
| Molecular weight (incorporated) | 329.20 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830778 | bulk |
| M830778-50UMOL | 50 µmol |
| M830778-100UMOL | 100 µmol |
| M830778-250MG | 250 mg |
| M830778-1000MG | 1 g |

8. Non-constrained Nucleic Acid

Acronym: NNA

Non-constrained Nucleic Acid (NNA) is an artificial acyclic phosphodiester DNA analogue composed of stereoisomerically pure (S,S)-2-(nucleobase-methyl)butane-1,3-diols.



The (S,S)-stereoisomer of NNA forms homoduplexes of greater stability than DNA oligonucleotides of the same sequences and does not hybridize with natural DNA to form heteroduplexes (Ref. 33). For a 10-12-mer, on average, the NNA homoduplexes' melting temperature (T_m) is 8°C higher than the respective DNA (Figure 9) and about 8°C lower than the p-DNA duplex depending on nucleobase composition.

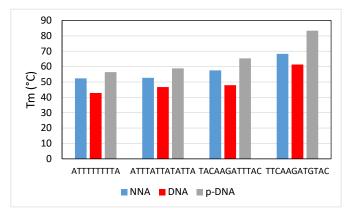


Figure 9 Melting temperature (T_m) comparison of NNA, DNA and p-DNA duplexes of different nucleobase composition.

NNA, as a tuned down alternative to p-DNA, is also not recognizable by DNA processing enzymes and is useful for labeling, barcoding or anchoring of multiple DNA-containing substrates co-existing in one mixture, array, or lateral flow strip.

| Available NNA products | Product number |
|------------------------|----------------|
| NNA T Phosphoramidite | M830775 |
| NNA A Phosphoramidite | M830772 |
| NNA C Phosphoramidite | M830773 |
| NNA G Phosphoramidite | M830774 |

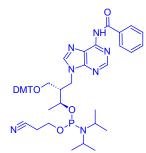
NNA T Phosphoramidite Product number: M830775; CAS #: 2378004-25-0



| Formula | C ₄₀ H ₅₁ N ₄ O ₇ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 730.83 Da |
| Molecular weight (incorporated) | 290.20 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830775 | bulk |
| M830775-50UMOL | 50 µmol |
| M830775-100UMOL | 100 µmol |
| M830775-250MG | 250 mg |
| M830775-1000MG | 1 g |

NNA A Phosphoramidite Product number: M830772; CAS #: 2378004-35-2

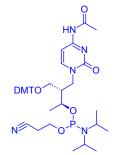


_

| Formula | C ₄₇ H ₅₄ N ₇ O ₆ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 843.95 Da |
| Molecular weight (incorporated) | 299.22 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830772 | bulk |
| M830772-50UMOL | 50 µmol |
| M830772-100UMOL | 100 µmol |
| M830772-250MG | 250 mg |
| M830772-1000MG | 1 g |

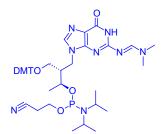
NNA C Phosphoramidite Product number: M830773; CAS #: 2378004-44-3



| Formula | C ₄₁ H ₅₂ N ₅ O ₇ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 757.85 Da |
| Molecular weight (incorporated) | 275.19 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalas aunthau | I for the stars |
|-----------------|-----------------|
| Catalog number | Unit size |
| M830773 | bulk |
| M830773-50UMOL | 50 µmol |
| M830773-100UMOL | 100 µmol |
| M830773-250MG | 250 mg |
| M830773-1000MG | 1 g |

NNA G Phosphoramidite Product number: M830774; CAS #: 2378004-41-0



| Formula | C ₄₃ H ₅₅ N ₈ O ₆ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 810.92 Da |
| Molecular weight (incorporated) | 315.22 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Multiple phosphoramidite additions and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830774 | bulk |
| M830774-50UMOL | 50 µmol |
| M830774-100UMOL | 100 µmol |
| M830774-250MG | 250 mg |
| M830774-1000MG | 1 g |

9. CDPI₃ MGB[™]-Oligonucleotide conjugates and their applications

The tripeptide of dihydropyrroloindole-carboxylate (CDPI₃) (Ref. 19) (Figure 10) is a minor groove binding (MGB) moiety derived from the natural product CC-1065 with strong DNA binding properties. CDPI₃ MGB is a crescent-shaped molecule which binds isohelically within the B-form DNA minor groove.

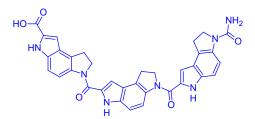


Figure 10 1,2-Dihydro-(3H)-pyrrolo[3,2-e]indole-7-carboxylate tripeptide (CDPI₃).

The reversible binding is mediated via hydrophobic and van der Waals interactions between the MGB and the floor of the groove. The MGB moiety occupies a region of duplex DNA approximately 5 bases long (Figure 11) and binds to both A/T and G/C rich sequences with association constants of $K_a \sim 1 \times 10^7 \, M^{-1}$ and $K_a \sim 1 \times 10^5 \, M^{-1}$, respectively.

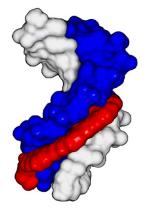


Figure 11 Connolly surface representation of a decamer DNA duplex formed between an MGB-ODN and its complement (Ref. 20). Red represents the MGB and linker moieties, blue is the MGB-labeled strand and light gray is the complement strand.

Synthetic oligonucleotides (ODNs) with covalently attached CDPI₃ MGB moieties were first introduced in 1995 by Lukhtanov et al. (Ref. 21). It has since been shown that such ODNs have enhanced DNA affinity and have improved the hybridization properties of sequence-specific DNA probes. Short MGB-oligonucleotides hybridize with single-stranded DNA to give more stable DNA duplexes than unmodified ODNs of similar length. For example, a DNA duplex formed between the CDPI₃ MGB-(dT)₈ conjugate and poly(dA) template has a melting temperature (T_m) that is 44°C higher than an unmodified duplex. Mismatch discrimination of short MGB-ODNs is also enhanced versus unmodified oligonucleotides. It was demonstrated that mismatches under the MGB binding region were more easily discriminated for a 15-mer MGB-ODN versus an unmodified ODN with up to a 3-fold increase in free energy difference (Ref. 22).

The tethered CDPI₃ MGB moiety has a preference for an A/T rich, B-form DNA duplex but is capable of binding to DNA duplexes with mixed sequences as well as to some modified backbone nucleic acids, as investigated by Kutyavin et al. (Ref. 23). The preference for A/T sequences has a useful practical application—it is well known that DNA duplex T_m depends on A/T and G/C content with A/T rich sequences being much less stable. CDPI₃ MGB tethering significantly reduces the difference, such that probes of equal length have similar T_m values regardless of base composition.

Applications

1. Arrest of primer extension and PCR blockers

CDPI₃ MGB-ODN conjugates were investigated for potential use as antigene agents via the inhibition of DNA polymerase (Ref. 24). The study, which was done in the context of a single-stranded DNA phage, demonstrated that T7 DNA polymerase was physically blocked when a complementary 16-mer 5'-CDPI₃ MGB-ODN was hybridized to a downstream site. Blockage was abolished when a single mismatch was introduced. A 16-mer with 3'-CDPI₃ MGB moiety also failed to arrest primer extension. The exceptional efficiency of the primer extension arrest was attributed to DNA polymerase's inability to displace the 5' end of the duplex super-stabilized with the CDPI₃ MGB group.

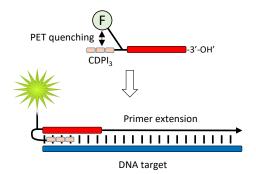
It has since been shown that 5'-CDPI₃MGB-ODNs are able to arrest Taq DNA polymerase and therefore can be used at PCR temperatures as well. This came as a surprise since it was expected that the 5' exonuclease Taq polymerase activity would degrade such duplexes. Evidently, 5'-MGB labeling makes ODNs resistant to 5' exonuclease digestion. Such ODNs can

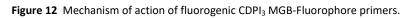
be used as PCR blockers to prevent amplification of selected DNA sequences.

2. Short and fluorogenic PCR primers

Efficient priming of PCR was demonstrated with 5'-MGB-ODNs as short as 8-mers using modified (touch-down) PCR cycling conditions or 10-16-mers using a regular PCR cycle (Ref. 25). The PCR was shown to produce specific amplification products of expected size. The reduced-length primers were suggested for use for PCR amplification of viral sequences which possess a high degree of variability or techniques such as gene hunting and differential display which amplify multiple sequences using short primer pairs.

5'-MGB-primers which also have an attached 5'-fluorophore are able to quench the dye fluorescence via the photo-induced electron transfer (PET) mechanism, depicted in Figure 12. Such primers are significantly quenched in a single strand state but become highly fluorescent when incorporated into the PCR amplicon allowing for detection of target amplification.





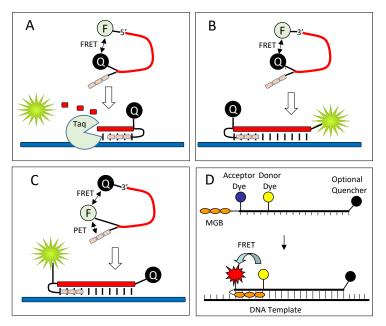
3. Real-time PCR probes

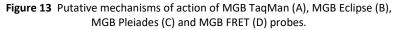
The stronger binding of MGB-ODNs versus unmodified ODNs allows for more stringent hybridization conditions to be used in DNA hybridization assays. Short MGB-ODNs with improved mismatch discrimination are especially useful in PCR assays since they bind efficiently and specifically during the high-temperature primer extension cycle. Several types of realtime PCR probes that utilize the MGB moiety have been developed.

• MGB TaqMan[®] probes (Ref. 22) have CDPI₃ MGB-Quencher and Fluorophore tethered to the 3' and 5' ends, respectively. Provided that specific sequence is present in the target DNA, the TaqMan

probes are degraded by Taq polymerase during PCR releasing unquenched fluorophore.

- MGB Eclipse® hybridization probes (Ref. 26) have the CDPI₃ MGB-Quencher and Fluorophore attached at the 5' and 3' ends, respectively. They are non-degradable and their fluorescence is strongly increased upon the probes' hybridization to amplified targets during the annealing step.
- MGB Pleiades[®] probes (Ref. 8) are also non-degradable hybridization probes. They have the CDPI₃ MGB-Fluorophore and Quencher moieties tethered to the 5' and 3' ends, respectively. These probes utilize the unique dual fluorescence quenching mechanism to significantly reduce background fluorescence and improve signal-to-background ratio.
- MGB FRET probes are similar to MGB Pleiades but contain an additional (donor) fluorophore conjugated to an internal nucleobase. MGB FRET probes allow for multicolor detection with a single wavelength excitation. MGB FRET probes offer high signalto-background ratios due to low background fluorescence via the dual quenching mechanism.





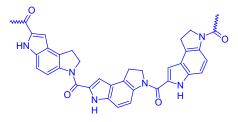
4. miRNA Inhibitors

In a recent US patent (Ref. 27), it was disclosed that MGB-ODNs with a 2'-OMe sugar-phosphate nucleic acid backbone are highly efficient and specific miRNA inhibitors. The inhibition is more pronounced when the minor groove binder is tethered to the 5'end of a miRNA inhibitor. The mechanism of this effect is not fully understood but could be attributed to the ability of 5'-MGB moiety to stabilize nucleic acid duplex and block enzymatic activities.

MGB-ODN-based miRNA inhibitors have also shown improved cellular uptake and promise as pharmaceuticals by modulating gene expression (Ref. 28).

CDPI₃ Minor Groove Binder

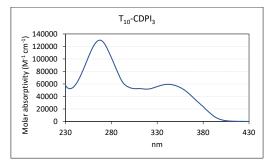
Acronym: CDPI₃ MGB



CDPI₃ MGB is an efficient duplexstabilizing agent. This ligand improves background fluorescence and mismatch discrimination abilities of DNA probes. When attached to the 5'-end of a probe it inhibits the 5' \rightarrow 3' exonuclease activity of DNA polymerases.

| Absorbance maximum* | 340 nm |
|---------------------------------|-------------------------------------------|
| Emission maximum* | N/A |
| Extinction coefficient (260 nm) | 37,900 M ⁻¹ cm ^{-1**} |
| Extinction coefficient (340 nm) | 59,300 M ⁻¹ cm ⁻¹ |

*Measured for T10-CDPI3 conjugate (50 mM Tris-HCl pH 8.5, 20°C) **CDPI3 contribution only

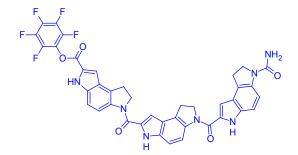


| Available CDPI ₃ MGB products | Product number |
|------------------------------------------------|-----------------------------------|
| CDPI ₃ MGB Terminal Phosphoramidite | M830252 |
| CDPI ₃ MGB CPG | M830739 |
| CDPI ₃ MGB Polystyrene*** | M830203 (bulk), M100103 (columns) |

***Suitable for ABI 3900 DNA synthesizer

CDPI₃ PFP ester

Product number: M830187

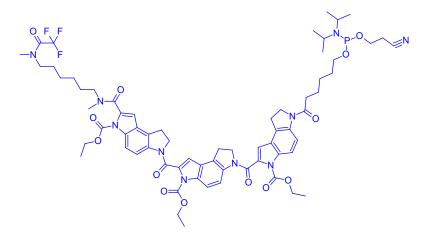


| Formula | $C_{40}H_{26}F_5N_7O_5$ |
|------------------------------------------|----------------------------|
| Molecular weight (reagent) | 779.67 Da |
| Molecular weight (incorporated) | 595.60 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830187 | bulk |
| M830187-1MG | 1 mg |
| M830187-10MG | 10 mg |

CDPI₃ MGB Terminal Phosphoramidite Product number: M830252

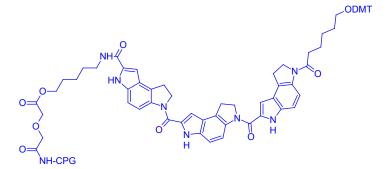


| Formula | $C_{67}H_{82}F_3N_{10}O_{13}P$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 1323.39 Da |
| Molecular weight (incorporated) | 872.94 Da |
| Purity (reverse phase HPLC) | <u>></u> 85% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity before/after coupling | ¹ H, ³¹ P NMR /Mass spectroscopy |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 9 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830252 | bulk |
| M830252-50UMOL | 50 µmol |
| M830252-100UMOL | 100 µmol |
| M830252-250MG | 250 mg |
| M830252-1000MG | 1 g |

CDPI₃ MGB CPG

Product number: M830739

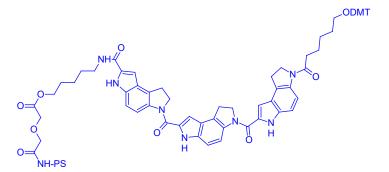


| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 831.87 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 2 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830739 | bulk |
| M830739-100MG | 100 mg |
| M830739-250MG | 250 mg |
| M830739-1000MG | 1 g |

CDPI₃ MGB Polystyrene

Product numbers: M830203 (bulk), M100103 (columns)

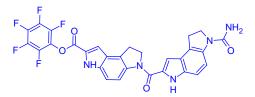


| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 831.87 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 6 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830203 | bulk |
| M100103-P3 | Pack of 3 columns* |
| M100103-P10 | Pack of 10 columns* |
| M100103-P30 | Pack of 30 columns* |
| M100103-P100 | Pack of 100 columns* |

CDPI₂ PFP ester

Product numbers: M830790



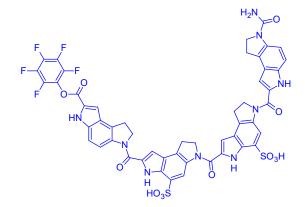
| Formula | C29H18E5N5O4 |
|------------------------------------------|--------------------------------|
| Molecular weight (reagent) | 595.48 Da |
| Molecular weight (incorporated) | 411.41 Da |
| Purity* (reverse phase HPLC) | >90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry∕ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830790 | bulk |
| M830790-1MG | 1 mg |
| M830790-10MG | 10 mg |

CDPI₄ (SO₃H)₂ PFP ester

Product numbers: M830188



| Formula | C ₆₉ H ₆₆ F ₅ N ₁₃ O ₁₂ S ₂ (bis-DBU salt) |
|------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 1428.47 Da (bis-DBU salt) |
| Molecular weight (incorporated) | 939.92 Da |
| Purity* (reverse phase HPLC) | <u>></u> 90% |
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830188 | bulk |
| M830188-1MG | 1 mg |
| M830188-10MG | 10 mg |

10. PBI₃ MGB[™]-Oligonucleotide conjugates

Piperazino-tris-benzimidazole (PBI₃) (Figure 14) is a DNA minor groove binder derived from the anthelmintic compound Hoechst 33258. It occupies a region of 5 to 6 base pairs in a DNA duplex and preferentially binds to A/T rich sequences.

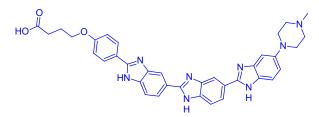


Figure 14 Structure of PBI₃ minor groove binder

Similar to CDPI₃, oligonucleotides with a conjugated PBI₃ moiety demonstrate enhanced hybridization properties (table below). While both types of MGB prefer A/T rich sequences, the degree of stabilization of such duplexes by PBI₃ is substantially higher, on average by 10°C for a 12-mer sequence with an A/T rich 5-6 base MGB binding region.

| 5'-3' | 5'-MGB* | | E' 2' | | PBI ₃ -CDPI ₃ |
|--------------------------|---------|---------------|-------|------|-------------------------------------|
| 5-3 | None | CDPI ₃ | PBI₃ | ΔTm | |
| TAAAAGGTGTAC | 38.5 | 53.9 | 63.2 | 9.3 | |
| TATAAG GTGTAC | 38.2 | 51.7 | 56.8 | 5.1 | |
| TATTTG GTGTAC | 40.8 | 58.0 | 67.3 | 9.2 | |
| TAAAAA TTGTAC | 34.3 | 52.2 | 66.4 | 14.3 | |
| TAAAAA GTGTAC | 34.7 | 52.8 | 63.2 | 10.4 | |
| ATTTTTTGTAC | 33.6 | 55.8 | 66.2 | 10.4 | |
| TTTTTTGTGTAC | 34.9 | 58.6 | 67.9 | 9.3 | |
| TTTTTGGTGTAC | 37.6 | 59.3 | 68.5 | 9.2 | |
| TACAAG GTGTAC | 41.9 | 48.8 | 53.1 | 4.4 | |
| TGCAAG GTGTAC | 46.8 | 52.2 | 54.8 | 2.6 | |
| TGCACG GTGTAC | 51.7 | 56.2 | 56.9 | 0.6 | |
| TGCACC GTGTAC | 52.1 | 56.4 | 57.3 | 0.9 | |
| Average T _m : | 40.1 | 55.1 | 62.4 | | |

Comparison of DNA duplex stabilization (duplex T_m (°C)) by conjugated CDPI₃ and PBI₃ minor groove binders

*Also contained FAM-HEG (M830100) between MGB and 5'-end. Highlighted are probable MGB binding regions.

Stabilization of G/C rich sequences is comparably modest for both MGB types, 1-5°C for a 12-mer sequence.

Another aspect of an MGB conjugate is the ability to distinguish between fully (matched) and partially (mismatched) complementary duplexes. With regards to match versus mismatch, both CDPI₃ and PBI₃ demonstrate similar performance. On average, about 11°C duplex destabilization (T_m decrease) is observed in a 12-mer sequence when a single mismatched base pair is introduced.

| Match-Mismatch (ΔT _m) | | | | |
|-----------------------------------|-------|------|------------------|----------|
| 5'-3' | CDPI₃ | PBI₃ | Mismatch | Position |
| TAAAAGGTGTAC | 12.9 | 15.0 | A/A | 3 |
| TAAAA <mark>G</mark> GTGTAC | 11.4 | 11.5 | G/T | 6 |
| TATAAGGTGTAC | 10.3 | 7.4 | T/T | 3 |
| TATTTGGTGTAC | 9.2 | 12.9 | A/A | 2 |
| TAAAAATTGTAC | 27.9 | 31.1 | A/C, T/C | 6,7 |
| TAAAAAGTGTAC | 11.8 | 12.9 | A/C | 6 |
| TAAAAAGTGTAC | 10.8 | 9.8 | G/A | 7 |
| ATTTTTTTGTAC | 10.2 | 8.4 | A/A, T/C | 1,7 |
| TTTTTTGTGTAC | 14.7 | 12.9 | T/C | 6 |
| TTTTTTGTGTAC | 18.7 | 14.8 | T/T <i>,</i> G/A | 1,7 |
| TTTTTGGTGTAC | 12.9 | 13.0 | T/T | 2 |
| TTTTT <mark>G</mark> GTGTAC | 11.2 | 10.2 | G/A | 6 |
| TACAAGGTGTAC | 8.9 | 8.8 | A/C | 2 |
| T <mark>G</mark> CAAGGTGTAC | 10.0 | 10.0 | G/T | 2 |
| TGCA <mark>C</mark> GGTGTAC | 17.5 | 14.5 | C/T | 5 |
| TGCAC <mark>G</mark> GTGTAC | 12.7 | 13.0 | G/G | 6 |
| TGCACCGTGTAC | 15.7 | 17.4 | C/C | 6 |
| Average ΔT_m : | 11.3 | 11.2 | _ | |

Comparison of mismatch discrimination by conjugated CDPI₃ and PBI₃ minor groove binders

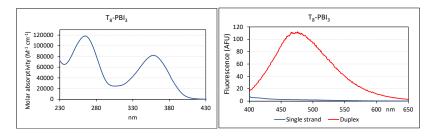
Despite the similarities, PBI₃ MGB is an attractive alternative to CDPI₃ when duplex hyper-stabilization is required, for instance, for a sequence specific polymerase arrest (PCR blocking).

Another distinctive feature of the PBI₃ moiety is its fluorescence properties. Its fluorescence efficiency increases significantly upon binding with a double-stranded DNA. In the case of a PBI₃-oligonucleotide conjugate, fluorescence increase is triggered by the conjugate hybridization with a complementary target. In principle, this allows for a very simple hybridization probe design that requires no additional fluorophore or a quencher.

Optical properties of conjugated PBI₃

| Absorbance maximum* | 358 nm |
|---------------------------------|--------------------------------------------|
| Emission maximum** | 473 nm |
| Extinction coefficient (260 nm) | 50,000 M ⁻¹ cm ^{-1***} |
| Extinction coefficient (358 nm) | 82,700 M ⁻¹ cm ⁻¹ |

*Measured for T₈-PBI₃ conjugate (50 mM Tris-HCl pH 8.5, 20°C) **Measured for 200 nM T₈-PBI₃ conjugate (10 mM Tris-HCl pH 8.9, 40 mM NaCl, 5 mM MgCl₂) at 20°C in the presence of 400 nM A₈C complement ***PBI₃ contribution only

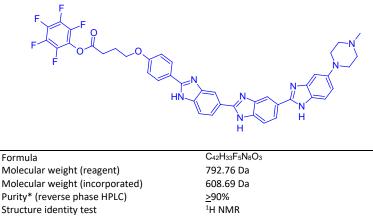


| Available PBI ₃ MGB products | Product number |
|-----------------------------------------|-----------------------------------|
| PBI ₃ MGB PFP ester | M830795 |
| PBI ₃ MGB CPG | M830796 |
| PBI₃ MGB Polystyrene**** | M830797 (bulk), M100243 (columns) |

****Suitable for ABI 3900 DNA synthesizer

PBI₃ MGB PFP ester

Product number: M830795



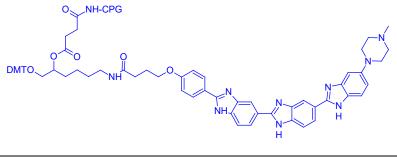
| | <u>~</u> 3070 |
|------------------------------------------|--------------------------------|
| Structure identity test | ¹ H NMR |
| Recommended coupling conditions | See conjugation protocol |
| Deprotection conditions | N/A |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 1 year |
| Available documents | CoA, conjugation protocols |

*Purity of this active derivative is verified by HPLC & NMR and usually exceeds 90%. Some loss of activity, however, may occur during packaging and storage, especially for small (1-10 mg) unit sizes.

| Catalog number | Unit size |
|----------------|-----------|
| M830795 | bulk |
| M830795-1MG | 1 mg |
| M830795-10MG | 10 mg |

PBI₃ MGB CPG

Product number: M830796

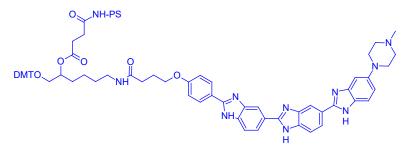


| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 803.84 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 55% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830796 | bulk |
| M830796-100MG | 100 mg |
| M830796-250MG | 250 mg |
| M830796-1000MG | 1 g |

PBI₃ MGB Polystyrene

Product numbers: M830797 (bulk), M100243 (columns)



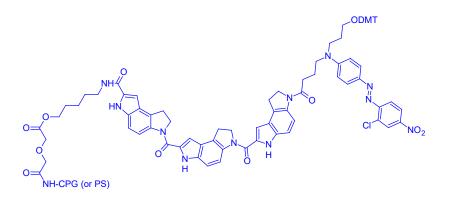
| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 803.84 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 55% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830797 | bulk |
| M100243-P3 | Pack of 3 columns* |
| M100243-P10 | Pack of 10 columns* |
| M100243-P30 | Pack of 30 columns* |
| M100243-P100 | Pack of 100 columns* |

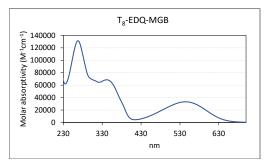
*ABI 3900 type columns, ~200 nmol/column

11. Eclipse Dark Quencher-MGB synthesis supports

Acronym: EDQ-MGB



EDQ-MGB CPG and polystyrene supports offer the most straightforward way of preparing MGB TagMan and MGB Eclipse probes (Figure 13A and 13B). When used in combination with 3'-nucleoside Figure phosphoramidites and a fluorophore phosphoramidite at the last step of the synthesis, the supports will yield a 5'-fluorophore, 3'-EDQ-MGB probe. which is the essence of the MGB TagMan probe's structure. Alternatively, the use of 5'-nucleoside phosphoramidites will afford a 3'-fluorophore, 5'-EDQ-MGB probe known as MGB Eclipse. MGB TagMan probes generate fluorescence signal during PCR amplification via 5'-exonuclease degradation in the presence of complementary targets. MGB Eclipse probes, on the other hand, are 5'-exonuclease resistant and remain annealed to complementary targets to generate fluorescent signal. Both types of probes benefit from the presence of the duplex-stabilizing MGB moiety which allows for shorter probes with improved mismatch discrimination and reduced background fluorescence.



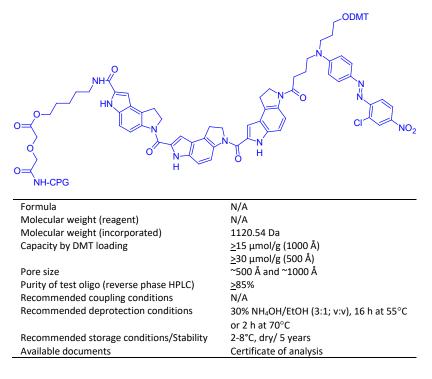
| Absorbance maximum* | 342 nm, 545 nm |
|---------------------------------|-------------------------------------------|
| Emission maximum* | N/A |
| Extinction coefficient (260 nm) | 55,700 M ⁻¹ cm ^{-1**} |
| Extinction coefficient (342 nm) | 68,700 M ⁻¹ cm ⁻¹ |
| Extinction coefficient (545 nm) | 33,300 M ⁻¹ cm ⁻¹ |

*Measured for T₈-EDQ-MGB conjugate (50 mM Tris-HCl pH 8.5, 20°C) **EDQ-MGB contribution only

| Available EDQ-MGB synthesis supports | Product number |
|--------------------------------------|-----------------------------------|
| EDQ-MGB Polystyrene | M830385 (bulk), M130008 (columns) |
| EDQ-MGB CPG (500 Å) | M830394 |
| EDQ-MGB CPG (1000 Å) | M830394-1000 |

EDQ-MGB CPG

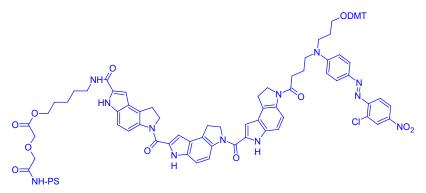
Product number: M830394 (500 Å), M830394-1000 (1000 Å)



| Catalog number | Unit size |
|---------------------|-----------|
| M830394 | bulk |
| M830394-100MG | 100 mg |
| M830394-250MG | 250 mg |
| M830394-1000MG | 1 g |
| M830394-1000 | bulk |
| M830394-1000-100MG | 100 mg |
| M830394-1000-250MG | 250 mg |
| M830394-1000-1000MG | 1 g |

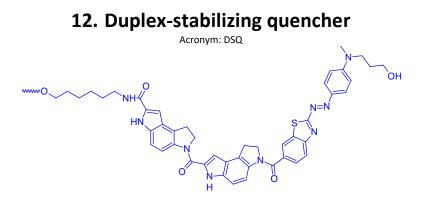
EDQ-MGB Polystyrene

Product number: M830385 (bulk), M130008 (columns)



| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 1120.54 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830385 | bulk |
| M130008-P3 | Pack of 3 columns* |
| M130008-P10 | Pack of 10 columns* |
| M130008-P30 | Pack of 30 columns* |
| M130008-P100 | Pack of 100 columns* |



DSQ is a single molecule that acts as an MGB and a universal fluorescence quencher at the same time. It was designed by converting a part of the original CDPI₃ MGB molecule into a quencher without compromising the MGB duplex-stabilizing properties. DSQ was developed to improve MGB TaqMan probe chemistry. It has a number of advantages over the EDQ-MGB chemistry. It eliminates the need for a separate MGB and quencher and streamlines probe manufacturing. More importantly, DSQ improves DNA duplex stabilization (Figure 16), fluorescence quenching (Figure 15 and Figure 17) and signal-to-background ratio (Figure 17).

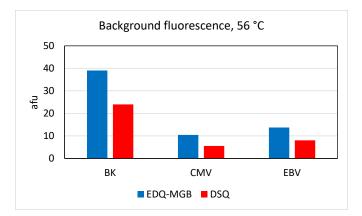


Figure 15 Comparison of background fluorescence for unhybridized FAM-labeled EDQ-MGB and DSQ probes. Examples include probes specific for BK virus (BK), cytomegalovirus (CMV), and Epstein-Barr Virus (EBV).

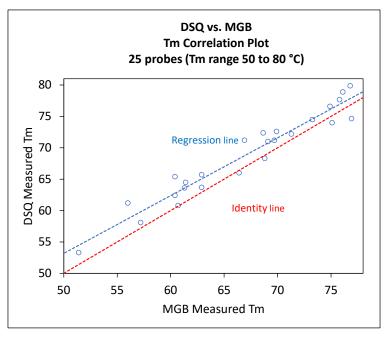


Figure 16 Comparison of melting temperatures of DSQ and EDQ-MGB probes.

On average, DSQ-probe/target duplexes are 2°C (range -2 to +5°C) more stable than the respective MGB-probe/target duplexes (Figure 16).

When compared to MGB TaqMan, DSQ TaqMan probes show a 20-50% reduction of baseline fluorescence, comparable amplification signals (and Ct values) and 10-100% increase of signal-to-baseline ratio (Figure 17).

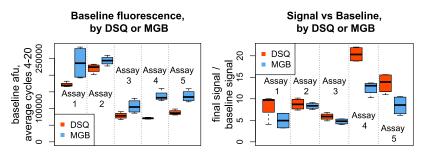
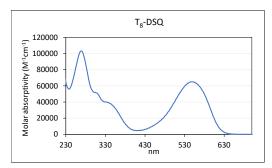


Figure 17 Comparison of real-time PCR baseline fluorescence and signal-tobaseline ratios of DSQ- and EDQ-MGB-modified probes.

Optical properties of conjugated DSQ

| Absorbance maximum* | 330 nm, 549 nm | |
|---------------------------------|--------------------------------------------|--|
| Emission maximum* | N/A | |
| Extinction coefficient (260 nm) | 25,000 M ⁻¹ cm ⁻¹ ** | |
| Extinction coefficient (330 nm) | 39,500 M ⁻¹ cm ⁻¹ | |
| Extinction coefficient (549 nm) | 65,000 M ⁻¹ cm ⁻¹ | |

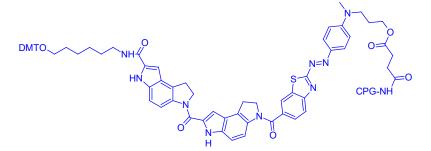
*Measured for T_8-DSQ conjugate (50 mM Tris-HCl pH 8.5, 20°C) **DSQ contribution only



| Available DSQ products | Product number |
|------------------------|-----------------------------------|
| DSQ Polystyrene | M830756 (bulk), M100501 (columns) |
| DSQ CPG | M830762 |

DSQ CPG

Product number: M830762

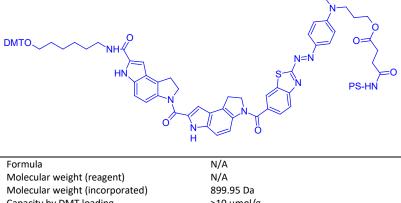


| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 899.95 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830762 | bulk |
| M830762-100MG | 100 mg |
| M830762-250MG | 250 mg |
| M830762-1000MG | 1 g |

DSQ Polystyrene

Product number: M830756 (bulk), M100501 (columns)



 Capacity by DMT loading
 ≥10 µmol/g

 Purity of test oligo (reverse phase HPLC)
 ≥65%

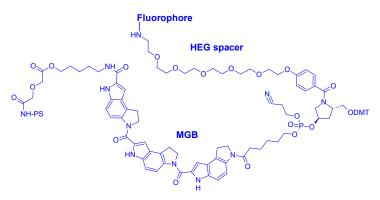
 Recommended coupling conditions
 N/A

 Recommended deprotection conditions
 30% NH₄OH/EtOH (or 2 h at 70°C

Recommended storage conditions/Stability Available documents 899.95 Da
≥10 μmol/g
≥65%
N/A
30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C
2-8°C, dry/ 5 years
Certificate of analysis

| Catalog number | Unit size |
|----------------|----------------------|
| M830756 | bulk |
| M100501-P3 | Pack of 3 columns* |
| M100501-P10 | Pack of 10 columns* |
| M100501-P30 | Pack of 30 columns* |
| M100501-P100 | Pack of 100 columns* |

13. Fluorophore-MGB (CDPI₃) Polystyrene supports

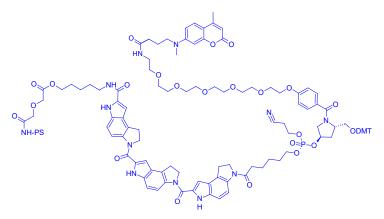


Fluorophore-MGB polystyrene supports have been developed for simultaneous incorporation of a fluorophore and the MGB residue either at the 3' or 5' end of an oligonucleotide. When used in combination with 5'-nucleoside phosphoramidites and a non-fluorescent guencher, these supports yield 5'-fluorophore-MGB, 3'-quencher oligonucleotide probes known as MGB Pleiades probes (Figure 13C). MGB Pleiades are hybridization-triggered fluorogenic probes, which are resistant to 5'exonuclease degradation and thus suitable for post-PCR melting curve analysis. Owing to the efficiency of a dual guenching mechanism, mediated by both the MGB and a non-fluorescent guencher, Pleiades probes possess a very low background fluorescence in the absence of a target. Fluorescence is restored upon probe hybridization with complementary signal-to-background target with excellent ratios. Additionally, fluorophores are separated from the MGB group by the hexa-ethylene glycol (HEG) spacer for improved hybridization-dependent fluorescence.

| Available Fluorophore MGB Polystyrenes | Product number |
|----------------------------------------|-----------------------------------|
| EB380-MGB Polystyrene | M830740 (bulk), M100431 (columns) |
| FAM-MGB Polystyrene | M830693 (bulk), M100185 (columns) |
| AP525-MGB Polystyrene | M830692 (bulk), M100186 (columns) |
| AP559-MGB Polystyrene | M830689 (bulk), M100181 (columns) |
| AP593-MGB Polystyrene | M830691 (bulk), M100135 (columns) |
| AP639-MGB Polystyrene | M830738 (bulk), M100293 (columns) |
| AP642-MGB Polystyrene | M830200 (bulk), M100153 (columns) |
| AP662-MGB Polystyrene | M830695 (bulk), M100250 (columns) |
| AP690-MGB Polystyrene | M830754 (bulk), M100499 (columns) |

EB380-MGB Polystyrene

Product number: M830740 (bulk), M100431 (columns)

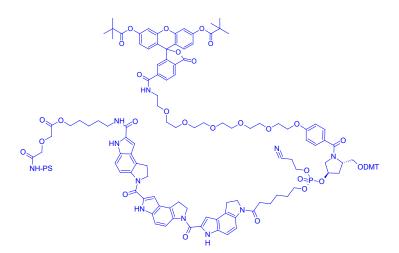


| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 1651.68 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 75% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 6 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|----------------|----------------------|
| M830740 | bulk |
| M100431-P3 | Pack of 3 columns* |
| M100431-P10 | Pack of 10 columns* |
| M100431-P30 | Pack of 30 columns* |
| M100431-P100 | Pack of 100 columns* |

FAM-MGB Polystyrene

Product number: M830693 (bulk), M100185 (columns)



| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 1752.69 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 75% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 8 years |
| Available documents | Certificate of analysis |

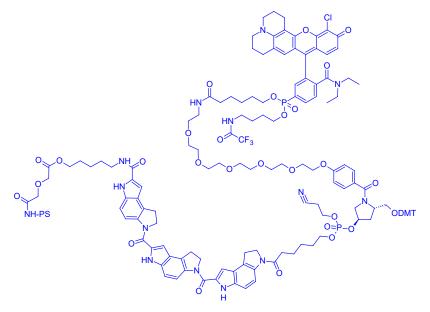
| Catalog number | Unit size |
|----------------|----------------------|
| M830693 | bulk |
| M100185-P3 | Pack of 3 columns* |
| M100185-P10 | Pack of 10 columns* |
| M100185-P30 | Pack of 30 columns* |
| M100185-P100 | Pack of 100 columns* |

| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2076.84 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 75% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 6 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|----------------|----------------------|
| M830692 | bulk |
| M100186-P3 | Pack of 3 columns* |
| M100186-P10 | Pack of 10 columns* |
| M100186-P30 | Pack of 30 columns* |
| M100186-P100 | Pack of 100 columns* |

AP559-MGB Polystyrene

Product number: M830689 (bulk), M100181 (columns)



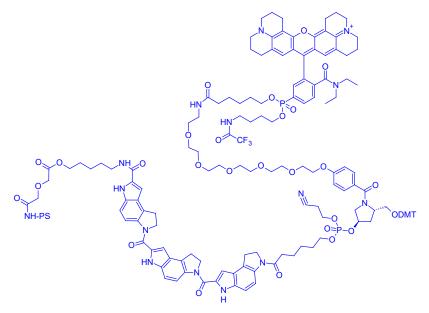
| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2071.52 Da |
| Capacity by DMT loading | ≥10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>≥</u> 65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830689 | bulk |
| M100181-P3 | Pack of 3 columns* |
| M100181-P10 | Pack of 10 columns* |
| M100181-P30 | Pack of 30 columns* |
| M100181-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

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AP593-MGB Polystyrene Product number: M830691 (bulk), M100135 (columns)

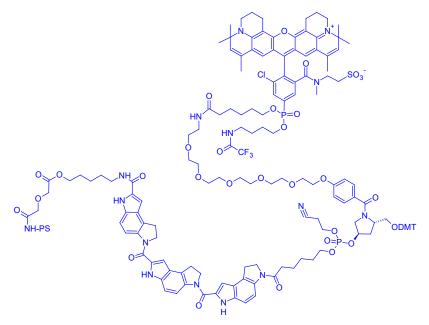


| Formula | N/A |
|-------------------------------------------|-----------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2116.22 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | >65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 4 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830691 | bulk |
| M100135-P3 | Pack of 3 columns* |
| M100135-P10 | Pack of 10 columns* |
| M100135-P30 | Pack of 30 columns* |
| M100135-P100 | Pack of 100 columns* |

AP639-MGB Polystyrene

Product number: M830738 (bulk), M100293 (columns)

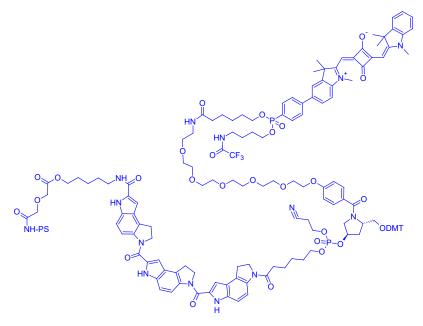


| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2296.82 Da |
| Capacity by DMT loading | >10 µmol/g |
| Purity of test oligo (reverse phase HPLC) | >65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH4OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 6 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830738 | bulk |
| M100293-P3 | Pack of 3 columns* |
| M100293-P10 | Pack of 10 columns* |
| M100293-P30 | Pack of 30 columns* |
| M100293-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

AP642-MGB Polystyrene Product number: M830200 (bulk), M100153 (columns)

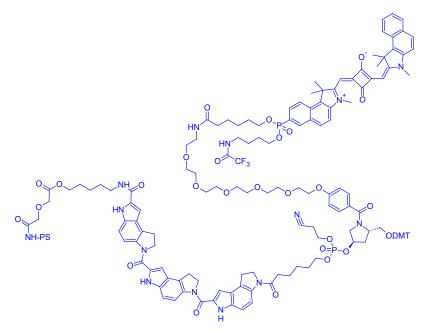


| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2071.14 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 8 years |
| Available documents | Certificate of analysis |
| | · · |

| Catalog number | Unit size |
|----------------|----------------------|
| M830200 | bulk |
| M100153-P3 | Pack of 3 columns* |
| M100153-P10 | Pack of 10 columns* |
| M100153-P30 | Pack of 30 columns* |
| M100153-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

AP662-MGB Polystyrene Product number: M830695 (bulk), M100250 (columns)



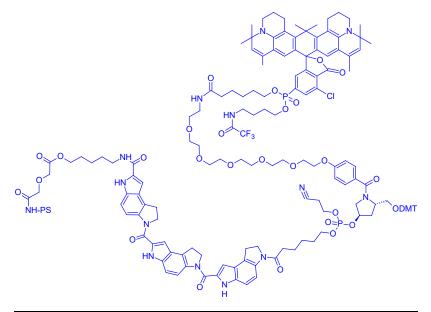
| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2095.16 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 9 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830695 | bulk |
| M100250-P3 | Pack of 3 columns* |
| M100250-P10 | Pack of 10 columns* |
| M100250-P30 | Pack of 30 columns* |
| M100250-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

AP690-MGB Polystyrene

Product number: M830754 (bulk), M100499 (columns)



| Formula | N/A |
|-------------------------------------------|-------------------------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 2201.75 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 65% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 3 years |
| Available documents | Certificate of analysis |

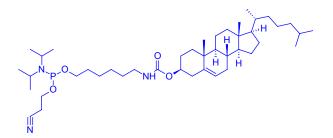
| Catalog number | Unit size |
|----------------|----------------------|
| M830754 | bulk |
| M100499-P3 | Pack of 3 columns* |
| M100499-P10 | Pack of 10 columns* |
| M100499-P30 | Pack of 30 columns* |
| M100499-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 704.91 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830761 | bulk |
| M830761-100MG | 100 mg |
| M830761-250MG | 250 mg |
| M830761-1000MG | 1 g |

Cholesterol Phosphoramidite Product number: M830785; CAS #: 143723-64-2



| Formula | C43H76N3O4P |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 730.06 Da |
| Molecular weight (incorporated) | 591.80 Da |
| Purity (reverse phase HPLC) | <u>≥</u> 90% |
| Purity of test oligo (reverse phase HPLC) | |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

-

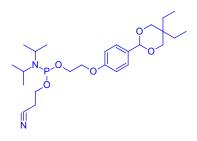
| Catalog number | Unit size |
|-----------------|-----------|
| M830785 | bulk |
| M830785-50UMOL | 50 µmol |
| M830785-100UMOL | 100 µmol |
| M830785-250MG | 250 mg |
| M830785-1000MG | 1 g |

15. Amine-modifiers, Linkers, and Spacers

| Product number | Product name | Functional group introduced |
|--------------------------------------|-----------------------------------------|---------------------------------------------|
| M830547 | Benzaldehyde Phosphoramidite | Aromatic aldehyde |
| M830758 | DMT-Hexanol Diglycolate-CPG | C6-Primary hydroxyl |
| M126137 (bulk), M100457 (colunms) | DMT-Hexanol-Diglycolate-Polystyrene | C6-Primary hydroxyl |
| M830780 | TFA-Aminopropyl-dU 3'- | C3-Primary amine at 5- |
| 101030700 | Phosphoramidite | position of Uracil |
| M830715 | TFA-Aminopropyl-dU 5'- | C3-Primary amine at 5- |
| 101050715 | Phosphoramidite | position of Uracil |
| M830784 | TFA-Aminopropyl-Super G 3'- | C3-Primary amine of |
| 101830784 | Phosphoramidite | Super G base |
| M830767 | Fmoc-HEG Phosphoramidite | Primary amine with a 17 atom spacer |
| M830769 | Fmoc-Hydroxyprolinol Phosphoramidite | Secondary amine |
| M830768 | Fmoc-Hydroxyprolinol CPG | Secondary amine |
| M830770 (bulk), M100510 (columns) | Fmoc-Hydroxyprolinol Polystyrene | Secondary amine |
| M830765 | Fmoc-Aminohexanediol CPG | C6-Primary amine |
| M830766 (bulk), M100511 (columns) | Fmoc-Aminohexanediol Polystyrene | C6-Primary amine |
| M830798 (bulk), M100508 (columns) | Fmoc-HEG-MGB Polystyrene | MGB and Primary amine with a 17 atom spacer |

Benzaldehyde Phosphoramidite

Product number: M830547; CAS #: 433684-36-7



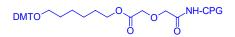
Benzaldehyde phosphoramidite is suitable for the introduction of an aromatic aldehyde at the 5' or 3' end of an oligonucleotide. The cyclic acetal provides a hydrophobic handle for reverse phase HPLC purification and can be removed after HPLC by treatment with 80% acetic acid. Alternatively, the acetal protection is removed during the standard on-line detritylation step.

Aromatic aldehydes are mild electrophiles, which are capable of forming Schiff base type adducts with various nucleophiles, e.g., amines, hydrazines or hydrazides (Ref. 34).

| Formula | $C_{25}H_{41}N_2O_5P$ |
|-------------------------------------------|---------------------------------------------------------------|
| Molecular weight (reagent) | 480.58 Da |
| Molecular weight (incorporated) | 228.13 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | <u><</u> -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Unit size |
|-----------|
| bulk |
| 50 µmol |
| 100 µmol |
| 250 mg |
| 1 g |
| |

DMT-Hexanol Diglycolate CPG Product number: M830758

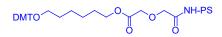


| Formula | N/A |
|-------------------------------------------|------------------------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 180.13 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830758 | bulk |
| M830758-100MG | 100 mg |
| M830758-250MG | 250 mg |
| M830758-1000MG | 1 g |

DMT-Hexanol Diglycolate Polystyrene

Product number: M126137 (bulk), M100457 (columns)

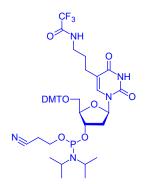


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 180.13 Da |
| Capacity by DMT loading | <u>≥</u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M126137 | bulk |
| M100457-P3 | Pack of 3 columns* |
| M100457-P10 | Pack of 10 columns* |
| M100457-P30 | Pack of 30 columns* |
| M100457-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

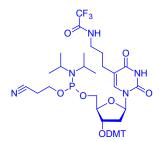
TFA-Aminopropyl-dU 3'-Phosphoramidite Product number: M830780; CAS #: 134140-86-6



| Formula | C44H53F3N5O9P |
|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 883.89 Da |
| Molecular weight (incorporated) | 347.26 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | Pre-treatment with 5% N,N,N',N'- tetramethylguanidine in CH ₃ CN followed by 30% NH ₄ OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830780 | bulk |
| M830780-50UMOL | 50 µmol |
| M830780-100UMOL | 100 µmol |
| M830780-250MG | 250 mg |
| M830780-1000MG | 1 g |

TFA-Aminopropyl-dU 5'-Phosphoramidite Product number: M830715; CAS #: 1146714-75-1

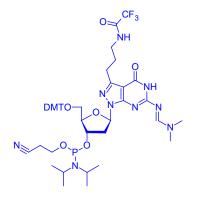


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| Formula | C44H53F3N5O9P |
|-------------------------------------------|-----------------------------------------------------|
| Molecular weight (reagent) | 883.89 Da |
| Molecular weight (incorporated) | 347.26 Da |
| Purity (reverse phase HPLC) | <u>></u> 80% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 80% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and |
| | extended coupling time |
| Recommended deprotection conditions | Pre-treatment with 5% N,N,N',N'- |
| | tetramethylguanidine in CH ₃ CN followed |
| | by 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | ≤ -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830715 | bulk |
| M830715-50UMOL | 50 µmol |
| M830715-100UMOL | 100 µmol |
| M830715-250MG | 250 mg |
| M830715-1000MG | 1 g |

TFA-Aminopropyl-Super G 3'-Phosphoramidite Product number: M830784; CAS #: 872840-74-9



| Formula | C48H59F3N9O8P |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular weight (reagent) | 978.01 Da |
| Molecular weight (incorporated) | 386.30 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | Pre-treatment with 5% N,N,N',N'- tetramethylguanidine in CH ₃ CN followed by 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830784 | bulk |
| M830784-50UMOL | 50 µmol |
| M830784-100UMOL | 100 µmol |
| M830784-250MG | 250 mg |
| M830784-1000MG | 1 g |
| | |

Fmoc-Hydroxyprolinol Phosphoramidite

Product number: M830769; CAS #: 2378004-37-4



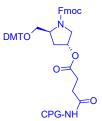
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| Formula | C ₅₀ H ₅₆ N ₃ O ₇ P |
|-------------------------------------------|-----------------------------------------------------------------|
| Molecular weight (reagent) | 841.97 Da |
| Molecular weight (incorporated) | 179.11 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and |
| | extended coupling time |
| Recommended deprotection conditions | Pre-treatment with 5% N,N,N',N'- |
| | tetramethylguanidine in CH₃CN followed |
| | by 30% NH₄OH/EtOH (17:3, v:v), 16 h at |
| | 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | < -10°C, dry/ 5 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size |
|-----------------|-----------|
| M830769 | bulk |
| M830769-50UMOL | 50 µmol |
| M830769-100UMOL | 100 µmol |
| M830769-250MG | 250 mg |
| M830769-1000MG | 1 g |

Fmoc-Hydroxyprolinol CPG Product number: M830768



| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 179.11 Da |
| Capacity by DMT loading | <u>></u> 15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830768 | bulk |
| M830768-100MG | 100 mg |
| M830768-250MG | 250 mg |
| M830768-1000MG | 1 g |

Fmoc-Hydroxyprolinol Polystyrene Product number: M830770 (bulk), M100510 (columns)



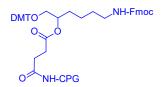
| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 179.11 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|----------------------|
| M830770 | bulk |
| M100510-P3 | Pack of 3 columns* |
| M100510-P10 | Pack of 10 columns* |
| M100510-P30 | Pack of 30 columns* |
| M100510-P100 | Pack of 100 columns* |

*ABI 3900 type columns, ~200 nmol/column

Fmoc-Aminohexanediol CPG

Product number: M830765

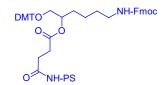


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 195.15 Da |
| Capacity by DMT loading | ≥15 μmol/g |
| Pore size | ~1000 Å |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ TBD |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|----------------|-----------|
| M830765 | bulk |
| M830765-100MG | 100 mg |
| M830765-250MG | 250 mg |
| M830765-1000MG | 1 g |

Fmoc-Aminohexanediol Polystyrene

Product number: M830766 (bulk), M100511 (columns)

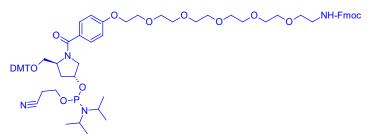


| Formula | N/A |
|-------------------------------------------|------------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 195.15 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size | |
|----------------|----------------------|--|
| M830766 | bulk | |
| M100511-P3 | Pack of 3 columns* | |
| M100511-P10 | Pack of 10 columns* | |
| M100511-P30 | Pack of 30 columns* | |
| M100511-P100 | Pack of 100 columns* | |

*ABI 3900 type columns, ~200 nmol/column

Fmoc-HEG Phosphoramidite Product number: M830767; CAS #: 2378004-40-9

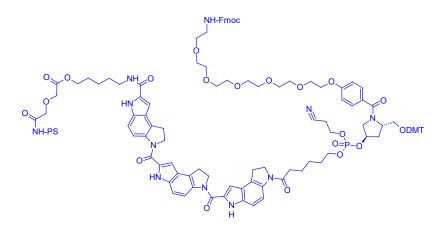


| Formula | C ₆₉ H ₈₅ N ₄ O ₁₄ P |
|-------------------------------------------|------------------------------------------------------------------|
| Molecular weight (reagent) | 1225.41 Da |
| Molecular weight (incorporated) | 562.54 Da |
| Purity (reverse phase HPLC) | <u>></u> 90% |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Identity | ¹ H, ³¹ P NMR |
| Recommended coupling conditions | Double phosphoramidite addition and extended coupling time |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C |
| Recommended storage conditions/Stability | <-10°C, dry/ 5 years |
| Available documents | Certificate of analysis |

| Catalog number | Unit size |
|-----------------|-----------|
| M830767 | bulk |
| M830767-50UMOL | 50 µmol |
| M830767-100UMOL | 100 µmol |
| M830767-250MG | 250 mg |
| M830767-1000MG | 1 g |

Fmoc-HEG-MGB Polystyrene

Product number: M830798 (bulk), M100508 (columns)



| Formula | N/A |
|-------------------------------------------|-----------------------------------------|
| Molecular weight (reagent) | N/A |
| Molecular weight (incorporated) | 1394.39 Da |
| Capacity by DMT loading | <u>></u> 10 μmol/g |
| Purity of test oligo (reverse phase HPLC) | <u>></u> 85% |
| Recommended coupling conditions | N/A |
| Recommended deprotection conditions | 30% NH₄OH/EtOH (3:1; v:v), 16 h at 55°C |
| | or 2 h at 70°C |
| Recommended storage conditions/Stability | 2-8°C, dry/ 5 years |
| Available documents | Certificate of analysis |
| | |

| Catalog number | Unit size | |
|----------------|----------------------|--|
| M830798 | bulk | |
| M100508-P3 | Pack of 3 columns* | |
| M100508-P10 | Pack of 10 columns* | |
| M100508-P30 | Pack of 30 columns* | |
| M100508-P100 | Pack of 100 columns* | |

*ABI 3900 type columns, ~200 nmol/column

Appendix A. Determination of the relative hydrophobicities of conjugated dyes using C18 column chromatography

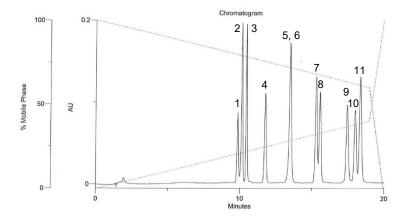


Figure 18 HPLC analysis of a mixture of fluorescent dye calibrators.

1 - T_8 -RR (M830731); 2 - T_8 -AP525 (M300587); 3 - T_8 -FAM-HEG (M300586); 4 - T_8 -EB380-HEG (M300585); 5 - T_8 -YY (M830730); 6 - T_8 -AP559 (M301430); 7 - T_8 -AP570 (M830700); 8 - T_8 -AP593 (M300392); 9 - T_8 -AP642 (M300589); 10 - T_8 -AP662 (M830724); 11 - T_8 -AP680 (M300753).

Column and HPLC conditions: C18 Luna (4.6x100 mm), Phenomenex (00D-4041-E0). Sample loop – 20 μ L. Flow rate – 1ml/min, Solvent A – 0.1 M triethylammonium acetate pH 7.5. Solvent B – CH₃CN. Gradient – 0-40% B in 19 min. Detection – 260 nm.

| Product number | Product name | Retention (min) | Hydrophobicity (% CH₃CN) |
|-------------------|------------------------------------------|--------------------|-----------------------------|
| M300585 | T ₈ -EB380-HEG Dye Calibrator | 11.77 | 22.0 |
| M300586 | T ₈ -FAM-HEG Dye Calibrator | 10.46 | 19.2 |
| M302132 | T8-GG Dye Calibrator | 11.53 | 21.5 |
| M300587 | T ₈ -AP525 Dye Calibrator | 10.14 | 18.6 |
| M830730 | T ₈ -YY Dye Calibrator | 13.49 | 25.6 |
| M301430 | T ₈ -AP559 Dye Calibrator | 13.49 | 25.6 |
| M830700 | T ₈ -AP570 Dye Calibrator | 15.30 | 29.4 |
| M830731 | T ₈ -RR Dye Calibrator | 9.85 | 17.9 |
| M300392 | T ₈ -AP593 Dye Calibrator | 15.56 | 29.9 |
| M301715 | T ₈ -AP639 Dye calibrator | 19.14 | 37.5 |
| M300589 | T ₈ -AP642 Dye Calibrator | 17.43 | 33.9 |
| M830724 | T ₈ -AP662 Dye Calibrator | 17.99 | 35.0 |
| M300753 | T ₈ -AP680 Dye Calibrator | 18.35 | 35.8 |
| M302123 | T ₈ -AP690 Dye Calibrator | 15.81 | 30.5 |

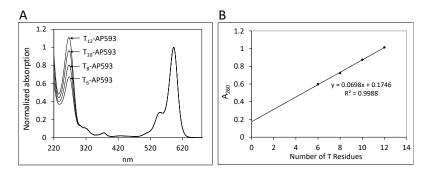
Appendix B. Determination of molar extinction coefficients of conjugated fluorophores and quenchers

The determination of extinction coefficient for a conjugated dye may be challenging due to the sensitivity of absorption and fluorescence spectra to the dye's environment and potentially complex dye-conjugate interactions. In the case of oligonucleotide conjugates, for instance, dyes may interact with nucleobases as well as other conjugated moieties. To take into account these effects, an optimal measurement procedure should employ a dye-oligonucleotide conjugate. However, determination of the dyeoligonucleotide concentration by pure conjugate mass, which is required for the direct extinction coefficient measurement, is often impractical. We have overcome this problem by using a concentration-independent approach which utilizes the known extinction coefficients of oligothymidylates and the dye absorption as an internal reference. In this approach, a series of dye-oligothymidylate conjugates is prepared with a variable number of thymidine units. The molar extinction coefficient for oligothymidylate can be described by a simple formula $E_{260} = 8100n + 600$, where n is the number of thymidine residues. Assuming that the dye conjugation does not affect this dependence, the combined extinction coefficient for dye-oligoT conjugate can be represented as E₂₆₀ (conjugate) $= 8100n + 600 + E_{260}(dye)$. To obtain $E_{260}(dye)$, one must measure $A_{260}(260)$ nm absorption), $A_{\lambda max}$ (dye) (absorption at the dye maximum) and plot $A_{260}(\text{conjugate})/A_{\lambda max}(\text{dye})$ values over the number of thymidine units. These data are then analyzed by linear regression to generate a line of best fit and its corresponding equation y = ax + b. Since T oligomers' slope parameter *a* equals 8100, the equation can be rewritten as y = 8100n + 1000b/a8100.

The procedure is illustrated in Figure 19. In this example, the described approach was used to determine an extinction coefficient for a T_n -conjugated AP593 dye.

It should be noted that the success of this method relies on several assumptions and requirements listed below:

- 1) Dye-oligonucleotide interactions are independent on the length of the oligomers;
- 2) All conjugates must be carefully purified to ensure accurate A₂₆₀ measurements;
- The linear fit of all data points should have a high (>0.99) R² value to ensure accurate y-intercept determination.

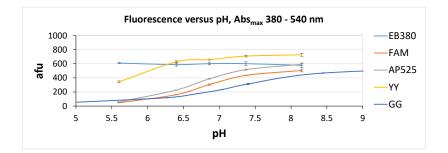


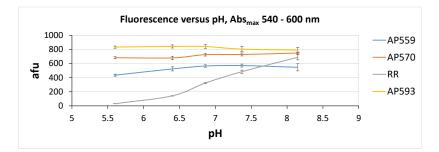
 $C E_{260}(dye) = 0.1746 * 8100 / 0.0698 - 600 = 19661.6 (M^{-1}cm^{-1})$

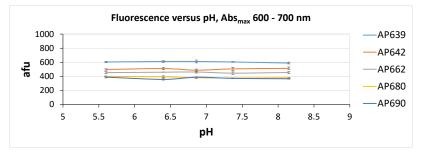
Figure 19 Extinction coefficient (E₂₆₀) determination for the AP593 dye conjugated to oligothymidylates.

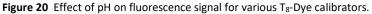
(A) Normalized (at 593 nm) absorption spectra of T_{12^-} , T_{3^-} and $T_{6^-}AP593$ conjugates. (B) Line of best fit (with equation and R^2 value) for the normalized A_{260} values versus number of thymidine units. (C) Calculation of the $E_{260}(dye)$ coefficient using the parameters a and b from the best fit linear equation. The y-intercept (b/a8100) of this line represents $E_{260}(dye) + 600$. Therefore, $E_{260}(dye) = b8100/a - 600$.

Appendix C. Effect of pH on dye fluorescence









Experimental conditions: Instrument: Varian Cary Eclipse; 5 nm slit width; Buffer: 40 mM sodium phosphate; Temperature: 20 °C.

Appendix D. Photostability of fluorescent dyes

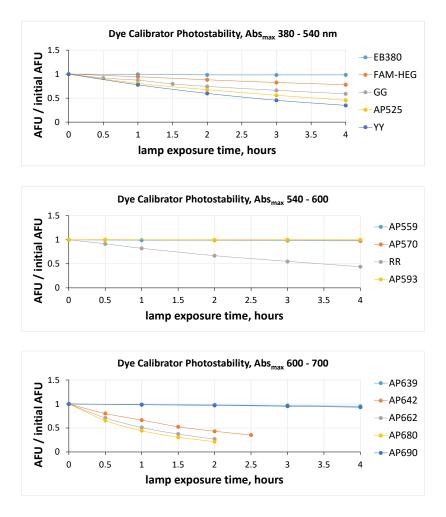
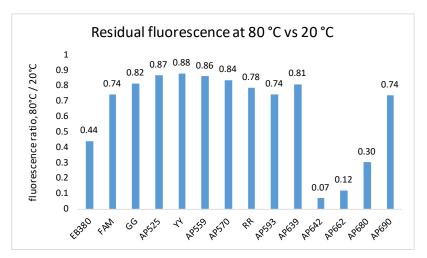


Figure 21 Relative photostability of fluorescent dyes.

500 nM dye calibrator solutions in TE (pH 8.0) were exposed to a 250 W incandescent bulb at a distance of 20 cm, using a water bath to maintain temperature at 20-35°C.



Appendix E. Fluorescence temperature dependence

Figure 22 Effect of temperature increase (from 20°C to 80°C) on dye fluorescence. *Concentration: 100 nM dye calibrator in 50 mM Tris-HCl (pH 8.0)*

QUICK REFERENCE GUIDE: FLUORESCENT DYES

| Name | Abs Max (nm) | E _{max} (M ⁻¹ cm ⁻¹) | Em Max (nm) | Φ | pH sensitivity | Hydro- phobicity* | Photo- stability** |
|------------------|--------------------|---------------------------------------------------------|----------------|------|----------------|----------------------|-----------------------|
| Epoch Blue 380 | 381 | 20,000 | 460 | 0.24 | not sensitive | 22 | High |
| 6-FAM | 496 | 80,400 | 517 | 0.88 | pKa~7 | 19.2 | Medium High |
| Gig Harbor Green | 497 | 93,200 | 521 | 0.85 | pKa~7.5 | 21.5 | Medium |
| AquaPhluor 525 | 527 | 99,300 | 549 | 0.73 | pKa~7 | 18.6 | Medium |
| Yakima Yellow | 525 | 86,500 | 547 | 0.57 | pKa~6 | 25.6 | Medium |
| AquaPhluor 559 | 559 | 86,500 | 581 | 0.64 | not sensitive | 25.6 | High |
| AquaPhluor 570 | 570 | 101,100 | 592 | 0.68 | not sensitive | 29.4 | High |
| Redmond Red | 580 | 64,500 | 594 | 0.46 | pKa~7.5 | 17.9 | Medium |
| AquaPhluor 593 | 593 | 115,240 | 613 | 0.61 | not sensitive | 29.9 | High |
| AquaPhluor 639 | 639 | 129,300 | 655 | 0.28 | not sensitive | 37.5 | High |
| AquaPhluor 642 | 642 | 283,200 | 653 | 0.13 | not sensitive | 33.9 | Low |
| AquaPhluor 662 | 662 | 241,100 | 672 | 0.14 | not sensitive | 35 | Low |
| AquaPhluor 680 | 682 | 221,300 | 704 | 0.07 | not sensitive | 35.8 | Low |
| AquaPhluor 690 | 689 | 141,900 | 709 | 0.14 | not sensitive | 30.5 | High |

*See Appendix A

**See Appendix D

QUICK GUIDE FOR OLIGONUCLEOTIDE SYNTHESIS: PHOSPHORAMIDITES

| | | | oupling ditions* | Recommended deprotection conditions | | |
|-----------------------------------|-------------------|---------------|---------------------|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------|--|
| Phosphoramidite name | Product number | # of addns | Wait time | | ^t BuNH ₂ :MeOH: H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C | |
| AP525 | M830199 | 2 | Standard | \checkmark | Not recommended | |
| AP525-HEG | M830714 | 2 | Standard | \checkmark | Not recommended | |
| AP559 internal | M830195 | 4 | Extended | \checkmark | \checkmark | |
| AP559 terminal | M830698 | 4 | Extended | \checkmark | \checkmark | |
| AP570 internal | M830675 | 2 | Standard | \checkmark | \checkmark | |
| AP570 terminal | M830699 | 2 | Standard | \checkmark | \checkmark | |
| AP593 | M830736 | 2 | Standard | \checkmark | \checkmark | |
| AP639 | M830741 | 4 | Extended | \checkmark | \checkmark | |
| AP690 | M830760 | 2 | Standard | Partial lactam formation | \checkmark | |
| Benzaldehyde | M830547 | 2 | Standard | \checkmark | \checkmark | |
| CDPI ₃ MGB Terminal | M830252 | 2 | Standard | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark | |
| Cholesterol | M830785 | 2 | Standard | ✓ | \checkmark | |
| EB380 | M830192 | 2 | Standard | \checkmark | \checkmark | |
| EB380-HEG | M830727 | 2 | Standard | \checkmark | \checkmark | |
| EDQ | M830028 | 2 | Standard | \checkmark | \checkmark | |
| EDQ-Super I 3'- | M830781 | 2 | Standard | \checkmark | \checkmark | |
| FAM | M100011 | 2 | Standard | \checkmark | \checkmark | |
| FAM-dU 3' | M830763 | 2 | Standard | \checkmark | \checkmark | |
| FAM-dU 5' | M830764 | 2 | Standard | \checkmark | \checkmark | |
| FAM-HEG | M830100 | 2 | Standard | \checkmark | \checkmark | |
| FAM-Super I 3'- | M830783 | 2 | Standard | \checkmark | \checkmark | |
| Fmoc-HEG | M830767 | 2 | Standard | \checkmark | \checkmark | |
| Fmoc- hydroxyprolinol | M830769 | 2 | Standard | ~ | ✓ | |
| LQ-380 Internal | M830786 | 2 | Standard | \checkmark | \checkmark | |
| LQ-400 Internal | M830788 | 2 | Standard | ✓ Partial dye degradation | ✓ | |
| NNA A | M830772 | 2 | Standard | \checkmark | \checkmark | |
| NNA C | M830773 | 2 | Standard | \checkmark | \checkmark | |
| NNA G | M830774 | 2 | Standard | \checkmark | \checkmark | |
| NNA T | M830775 | 2 | Standard | \checkmark | \checkmark | |
| p-DNA A | M830776 | 2 | Standard | \checkmark | \checkmark | |
| p-DNA C | M830777 | 2 | Standard | \checkmark | \checkmark | |
| p-DNA G | M830778 | 2 | Standard | \checkmark | \checkmark | |
| p-DNA T | M830779 | 2 | Standard | \checkmark | \checkmark | |
| Pyrene Internal | M830711 | 2 | Standard | \checkmark | \checkmark | |
| Pyrene-Super I- 3'- | M830782 | 2 | Standard | \checkmark | \checkmark | |
| Q-470 | M830565 | 2 | Standard | \checkmark | \checkmark | |
| Q-575 | M830566 | 2 | Standard | ✓ Partial dye degradation | \checkmark | |

| | | Coupling conditions* | | Recommended deprotection conditions | | |
|---------------------------------|-------------------|-------------------------|-----------|-------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|--|
| Phosphoramidite name | Product number | # of addns | Wait time | | ^t BuNH ₂ :MeOH: H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C | |
| Q-630 | M830567 | 2 | Standard | Not recommended | \checkmark | |
| Redmond Red | M830035 | 2 | Standard | \checkmark | \checkmark | |
| Super A 3'- | M830249 | 2 | Standard | \checkmark | ✓ Add extra time for multiple incorporations | |
| Super A 5'- | M830250 | 2 | Standard | V | Add extra time for multiple incorporations | |
| Super G 3'- | M830006 | 2 | Standard | \checkmark | \checkmark | |
| Super G 5'- | M830031 | 2 | Standard | \checkmark | \checkmark | |
| Super I 3'- | M830193 | 2 | Standard | ✓ TMG pre- treatment recommended | ✓ TMG pre- treatment recommended | |
| Super I 5'- | M830712 | 2 | Standard | ✓ TMG pre- treatment recommended | ✓ TMG pre- treatment recommended | |
| Super T 3'- | M830040 | 2 | Standard | \checkmark | \checkmark | |
| Super T 5'- | M830041 | 2 | Standard | \checkmark | \checkmark | |
| TFA-aminopropyl- dU 3' | M830780 | 2 | Standard | \checkmark | \checkmark | |
| TFA-aminopropyl- dU 5' | M830715 | 2 | Standard | ~ | \checkmark | |
| TFA-aminopropyl- Super G 3'- | M830784 | 2 | Standard | ~ | ✓ | |
| Yakima Yellow | M830552 | 2 | Standard | \checkmark | Not recommended | |

*Optimized for ABI 3900 DNA Synthesizer. Other instruments' coupling conditions may vary.

QUICK GUIDE FOR OLIGONUCLEOTIDE SYNTHESIS: SYNTHESIS SUPPORTS

| | Product number | Recommended deprot | tection conditions | |
|--------------|-------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|--|
| Product name | bulk (b) columns (c) | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C | |
| AP570 PS | M830677 (b) M100444 (c) | \checkmark | \checkmark | |
| AP525 CPG | M830671 | \checkmark | Not recommended | |
| AP525 PS | M830672 (b) M100276 (c) | \checkmark | Not recommended | |
| AP525-MGB PS | M830692 (b) M100186 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark | |
| AP559 CPG | M830673 | \checkmark | \checkmark | |
| AP559 PS | M830674 (b) M100278 (c) | \checkmark | √ | |
| AP559-MGB PS | M830689 (b) M100181 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark | |
| AP570 CPG | M830676 | \checkmark | \checkmark | |
| AP593 CPG | M830208 | \checkmark | \checkmark | |
| AP593 PS | M830732 (b) M100257 (c) | \checkmark | ~ | |
| AP593-MGB PS | M830691 (b) M100135 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark | |
| AP639 CPG | M830757 | \checkmark | ✓ | |
| AP639 PS | M830743 (b) M100440 (c) | \checkmark | \checkmark | |
| AP639-MGB PS | M830738 (b) M100293 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark | |
| AP642 CPG | M830190 | Not recommended | ✓ | |
| AP642 PS | M830678 (b) M100279 (c) M100280 (c) | Not recommended | \checkmark | |
| AP642-MGB PS | M830200 (b) M100153 (c) | Not recommended | ✓ | |
| AP662 CPG | M830191 | Not recommended | ✓ | |
| AP662 PS | M830679 (b) M100271 (c) | Not recommended | \checkmark | |
| AP662-MGB PS | M830695 (b) M100250 (c) | Not recommended | \checkmark | |
| AP680 CPG | M830680 | Not recommended | 50°C/8 h | |
| AP680 PS | M830681 (b) M100282 (c) | Not recommended | 50°C/8 h | |
| AP690 CPG | M830759 | Partial lactam formation | ~ | |

| | Product number | Recommended deprot | ection conditions |
|-----------------------------|----------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| Product name | bulk (b) columns (c) | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C |
| AP690 PS | M830755 (b) M100500 (c) | Partial lactam formation | ✓ |
| AP690-MGB PS | M830754 (b) M100499 (c) | Partial lactam formation | \checkmark |
| CDPI ₃ MGB CPG | M830739 | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark |
| CDPI ₃ MGB PS | M830203 (b) M100103 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark |
| Cholesterol CPG | M830761 | \checkmark | ✓ |
| DSQ CPG | M830762 | 30% NH₄OH/EtOH (3:1; v:v) | ✓ |
| DSQ PS | M830756 (b) M100501 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark |
| EB380 CPG | M830669 | \checkmark | \checkmark |
| EB380 PS | M830670 (b) M100441 (c) | \checkmark | ✓ |
| EB380-MGB PS | M830740 (b) M100431 (c) | 30% NH₄OH/EtOH (3:1; v:v) | ✓ |
| EDQ CPG | M830386 | \checkmark | \checkmark |
| EDQ PS | M830486 (b) M100447 (c) | \checkmark | ✓ |
| EDQ-MGB CPG (500 Å) | M830394 | 30% NH₄OH/EtOH (3:1; v:v) | ✓ |
| EDQ-MGB CPG (1000 Å) | M830394-1000 | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark |
| EDQ-MGB PS | M830385 (b) M130008 (c) | 30% NH₄OH/EtOH (3:1; v:v) | ✓ |
| FAM-HEG CPG | M830719 | \checkmark | \checkmark |
| FAM-HEG PS | M830718 (b) M100442 (c) | \checkmark | ✓ |
| FAM-MGB PS | M830693 (b) M100185 (c) | 30% NH₄OH/EtOH (3:1; v:v) | \checkmark |
| Fmoc-aminohexanediol CPG | M830765 | \checkmark | \checkmark |
| Fmoc-aminohexanediol PS | M830766 (b) M100511 (c) | \checkmark | ✓ |
| Fmoc-HEG-MGB PS | M830798 (b) M100508 (c) | \checkmark | ✓ |
| Fmoc-hydroxyprolinol CPG | M830768 | \checkmark | ✓ |
| Fmoc-hydroxyprolinol PS | M830770 (b) M100510 (c) | \checkmark | √ |
| Gig Harbor Green CPG | M830771 | \checkmark | ✓ |
| Gig Harbor Green PS | M400090 (b) M100433 (c) | √ | \checkmark |

| | Product number | Recommended deprot | ection conditions |
|--------------------------|----------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| Product name | bulk (b) columns (c) | 30% NH₄OH/EtOH (17:3, v:v), 16 h at 55°C or 2 h at 70°C | ^t BuNH ₂ :MeOH:H ₂ O (1:2:1, v:v:v), 16 h at 55°C or 3 h at 70°C |
| LQ-380 CPG | M830799 | \checkmark | \checkmark |
| LQ-380 PS | M830793 (b) M100505 (c) | \checkmark | ✓ |
| LQ-400 CPG | M830800 | ✓ Partial dye degradation | \checkmark |
| LQ-400 PS | M830794 (b) M100507 (c) | ✓ Partial dye degradation | \checkmark |
| PBI ₃ MGB CPG | M830796 | ✓ | ✓ |
| PBI₃ MGB PS | M830797 (b) M100243 (c) | \checkmark | ✓ |
| Q-470 CPG | M830562 | \checkmark | ✓ |
| Q-470 PS | M830568 (b) M100446 (c) | \checkmark | ✓ |
| Q-575 CPG | M830563 | ✓ Partial dye degradation | \checkmark |
| Q-575 PS | M830569 (b) M100448 (c) | ✓ Partial dye degradation | \checkmark |
| Q-630 CPG | M830564 | Not recommended | ✓ |
| Q-630 PS | M830570 (b) M100449 (c) | Not recommended | ✓ |
| Redmond Red CPG | M830390 | \checkmark | \checkmark |
| Redmond Red PS | M830729 (b) M100445 (c) | \checkmark | \checkmark |
| Yakima Yellow CPG | M830395 | \checkmark | Not recommended |
| Yakima Yellow PS | M400063 (b) M100443 (c) | √ | Not recommended |

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PRODUCT INDEX

| Product name | Product number | CAS # | Page |
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| | M830692 (bulk), | | 175 |
| AP525-MGB Polystyrene | M100186 (columns) | | 175 |
| | M830689 (bulk), | 204697-37-0 204697-37-0 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 2010 | 470 |
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| | M830738 (bulk), | | 170 |
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| · | M830754 (bulk), | | |
| AP690-MGB Polystyrene | M100499 (columns) | | 181 |
| AguaPhluor 492 NHS ester | M830194 | 2378004-15-8 | 17 |
| AquaPhluor 525 CPG | M830671 | | 34 |
| AguaPhluor 525 Internal | | | |
| Phosphoramidite | M830199 | 2378004-28-3 | 32 |
| AquaPhluor 525 Lactone | M830683 | 2378004-23-8 | 30 |
| | M830672 (bulk), | 2370004 23 0 | 50 |
| AquaPhluor 525 Polystyrene | M100276 (columns) | | 35 |
| AquaPhluor 525 Terminal | W100270 (columns) | | |
| Phosphoramidite | M830714 | 876746-10-0 | 31 |
| AquaPhluor 525-HEG Phosphoramidite | M100104 | 2270004 10 2 | 33 |
| AquaPhluor 559 CPG | M830673 | 2378004-19-2 | 44 |
| AquaPhluor 559 Internal | 101830073 | | 44 |
| Phosphoramidite | M830195 | 2378004-38-5 | 43 |
| Filosphoralindite | M830674 (bulk), | | |
| AquaPhluor 559 Polystyrene | M100278 (columns) | | 45 |
| AquaPhluor 559 Terminal | WI00278 (columns) | | |
| Phosphoramidite | M830698 | 2378004-43-2 | 42 |
| AquaPhluor 570 CPG | M830676 | | 49 |
| AquaPhluor 570 Internal | 101830676 | | 49 |
| • | M830675 | 2378004-20-5 | 48 |
| Phosphoramidite | M020677 (I. II.) | | |
| AquaPhluor 570 Polystyrene | M830677 (bulk), | | 50 |
| A | M100444 (columns) | | |
| AquaPhluor 570 Terminal | M830699 | 2378004-30-7 | 47 |
| Phosphoramidite | N1020106 | 11100000.000 | |
| AquaPhluor 593 PFP ester | M830196 | 1140966-99-9 | 57 |
| AquaPhluor 593 Terminal | M830736 | 2378004-17-0 | 58 |
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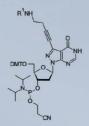
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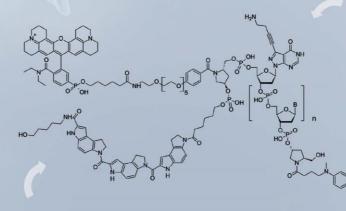
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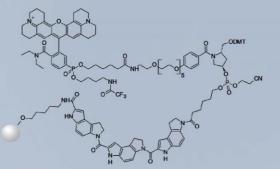


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