MGB Alert® Malaria Typer v2.0 RUO Detection Reagent

For Research Use Only. Not for use in diagnostic procedures.

SPIN TUBES PRIOR TO OPENING

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REF **M400874**

≤ -10°C

Intended Use

The **MGB Alert Malaria Typer v2.0 RUO Detection Reagent** is intended for use in a nucleic acid amplification test, to detect and distinguish *Plasmodium* species DNA in a nucleic acid sample. This product is intended for use with a real-time PCR system with appropriate optical specifications.

Assay Principle

The **MGB Alert Malaria Typer v2.0 RUO Detection Reagent** is a multiplex real-time PCR reagent designed with MGB Pleiades® hybridization probe chemistry to detect and distinguish DNA from ***Plasmodium falciparum, P. vivax, P. ovale,*** **and** ***P. malariae*** **or** ***knowlesi.*** To use this product effectively, thermal cycler parameters must include PCR thermal cycling with five color fluorescence detection. The reagent contains primers and probes specific to the 18S rRNA gene of *Plasmodium* species and a primer set and probe specific to an internal control (IC, sold separately). Probes are labeled with a fluorophore, a minor groove binder (MGB), and an Eclipse® Dark Quencher (EDQ).

The Pleiades hybridization probe chemistry in this product is unique. The fluorescence of unhybridized probes is quenched by a 5' MGB and a 3' EDQ. During each cycle of PCR, the primers and probes anneal to their DNA target template, if present, and a new DNA strand is synthesized from the primers by a polymerase. Hybridization of the probe to its target DNA separates the fluorophore from its quencher and MGB, allowing fluorescence emission. As the polymerase encounters the Pleiades probe annealed to the DNA template, the MGB on the 5' end of the probe blocks any 5' → 3'exonuclease activity of the polymerase, and the probe is displaced as DNA is synthesized. The PCR cycles result in exponential amplification of target DNA and, therefore, fluorescence levels. An optional dissociation stage results in exponential decrease of fluorescence levels, i.e., a melt curve, each characterized by a melt temperature (T_m).

Product Description

The **MGB Alert Malaria Typer v2.0 RUO Detection Reagent** is a ready-to-use 20X mix of primer and probe sets specific to the DNA of the target pathogens and to a synthetic sequence that serves as an IC to monitor assay performance. (The IC DNA template is sold separately, see Recommended Materials Not Provided.). Probes are labeled with FAM or an **AquaPhluor® (AP) fluorophore** (Table 1), and MGB and EDQ.

Table 1. MGB Alert Malaria Typer v2.0 RUO Detection Reagent components description. The number in the AP fluorophore name indicates its peak excitation wavelength.

Target	Probe fluorophore	Analogous fluorophore (for optical channel selection)
Plasmodium falciparum 18S rRNA gene	FAM	FAM
P. vivax 18S rRNA gene	AP642	Cy5, Quasar 670
P. ovale 18S rRNA gene	AP593	ROX, Texas Red
P. malariae or *P. knowlesi* 18S rRNA gene	AP525	VIC, JOE, HEX
Internal control IC2	AP690	Cy5.5, Quasar 705

The **MGB Alert Malaria Typer v2.0 RUO Detection Reagent** is provided at a volume of 120 µL and is designed to be combined with a master mix containing the necessary components for PCR (not provided). The 20X concentration is relative to the optimal final concentration of the primers and probes in the PCR.

Recommended Materials Not Provided

Table 2. Additional materials recommended for real-time PCR not provided in the MGB Alert Malaria Typer v2.0 RUO Detection Reagent.

Material	Use	Vendor	Part Number
MGB Alert® ELITaq Master Mix (2X)	Contains DNA polymerase, buffers, dNTPs, components for PCR	ELITechGroup	M800809, 48 reactions M800810, 480 reactions
Molecular biology grade water	Reaction mix preparation, negative controls	NA	NA
Positive controls	Positive control DNA for each pathogen target if available	NA	NA
Internal Control IC2 DNA	Internal control DNA template to monitor nucleic acid extraction and PCR performance	ELITechGroup	M800737

Recommended Reaction Setup

For optimal performance, protect all reagents from light, store at ≤-10°C while not in use, and limit the number of freeze-thaw cycles.

The following is an example of how to set up a real-time PCR using the MGB Alert Malaria Typer v2.0 RUO Detection Reagent for 50 µL reactions. Preparation of the reaction mix should be done in an area separate from preparation and addition of samples and controls.

Table 3. Example recipe for real-time PCR reaction mix.

Reagent	Stock concentration	Volume per reaction (µL)
PCR master mix	2X	25
Molecular biology grade water	--	12.5
MGB Alert RUO Detection Reagent	20X	2.5

Total reaction mix	--	40.0
Sample/control template	--	10.0

1. Prepare reaction mix as above (Table 3), or adjust volumes per reaction based on PCR master mix stock concentration and final reaction volume, multiplying the volumes per reaction by the number of samples + controls being run and an appropriate overage to add the needed dead volume (e.g., 20%).
2. Array 40 µL of the reaction mix into the wells of an optical plate or tubes.
3. Prepare positive and negative controls as appropriate.
4. Pipette 10 µL of sample or control into the appropriate well or tube containing reaction mix.
5. Seal the plate with optical adhesive film or cap PCR tubes.
6. Load the plate or tubes onto the real-time PCR instrument and program the thermal cycling as below (Table 4). Start the run.

Table 4. Recommended thermal cycling conditions. Adjustments may be required to optimize the PCR for various real-time PCR instruments. Refer to the instrument manual to set up the real-time PCR.

Stage		**Temperature**	**Time**
Denaturation	Hold	95°C	2 min
PCR (50 cycles)	Denaturation	95°C	5 sec
	Annealing*	56°C	20 sec
	Extension	76°C	20 sec
Dissociation (Optional)	Hold	95°C	15 sec
	Annealing	45°C	15 sec
	Melt*	45→80°C	Ramp at 0.06°C/sec

* Read fluorescence at the annealing stage of PCR and while ramping during the melt stage of dissociation.

Data Analysis Guidelines

Analysis of results from the MGB Alert Malaria Typer v2.0 RUO Detection Reagent should be performed for the PCR stage and optionally for the dissociation stage. Amplification of FAM, AP525, AP593, and/or AP642 fluorescence signals during PCR indicates the sample is positive for the DNA of the *Plasmodium* species identified by that fluorophore. (See Table 1.) AP525 identifies both *P. malariae* and *P. knowlesi* but does not distinguish them. The DNA of more than one species may be present in a nucleic acid sample. Amplification of the internal control AP690 signal indicates the PCR performed as expected. Amplification of the internal control AP690 signal may or may not be observed in samples that test positive for *Plasmodium* DNA, but must be observed in samples that test negative for *Plasmodium* DNA to ensure the PCR performed as expected.

Warnings and Precautions

- **This product is for Research Use Only, and not for use in diagnostic procedures.**
- Use of this product requires personnel trained in molecular biology techniques.
- This product shall be protected from light and stored at ≤-10°C while not in use.
- This product shall not be used after its expiration date.
- This product shall be used in accordance with local, state, and federal regulations or accreditation requirements.

- Disposal of all waste material shall be done in accordance with local, state, and federal regulations or accreditation requirements.

Technical Support

For technical support, call or email the ELITechGroup MDx (EG MDx) Technical Support Center: 1.800.453.2725 or mdx@elitechgroup.com, or contact your EG MDx Field Applications Specialist.

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Symbols

The following symbols are used within ELITechGroup MDx MGB Alert labeling

	Catalog number		Upper limit of temperature
	Lot or Batch Code		Expiration Date YYYY-MM-DD
	Manufacturer		Keep away from sunlight
 N	Contains sufficient for <N> tests		