

DSQ Alert™ *Cryptococcus* species RUO Detection Reagent

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

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PRIOR TO
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M400911



Intended Use

The **DSQ Alert™ *Cryptococcus* species RUO Detection Reagent** is intended for use in a nucleic acid amplification test, to detect and distinguish DNA from *Cryptococcus* species in a nucleic acid sample. This product is intended for use with a real-time PCR system with appropriate optical specifications.

Assay Principle

The **DSQ Alert *Cryptococcus* species RUO Detection Reagent** is a multiplex real-time PCR reagent designed with DSQ hybridization probe chemistry, the next evolution in MGB hydrolysis probes, to detect and distinguish DNA from ***Cryptococcus neoformans* and *Cryptococcus gattii***. To use this product effectively, thermal cycler parameters must include PCR thermal cycling with three color fluorescence detection. The reagent contains primers and probes specific to the mitochondrial gene for cytochrome b (cytb) of the *Cryptococcus* species labeled with a fluorophore and a duplex stabilizing quencher (DSQ). The reagent also contains a primer set and probe specific to an internal control (IC, sold separately).

The DSQ hybridization probe chemistry in this product is unique. During each cycle of PCR, the primers and probe anneal to their target template, if present, and DNA is synthesized from the primers by a polymerase. During synthesis, the polymerase encounters the probe annealed to the template downstream of the primer, and the exonuclease activity of the polymerase hydrolyzes the probe, releasing the fluorophore from the proximity of the DSQ and allowing fluorescence emission. The PCR cycles result in exponential amplification of the target DNA and fluorescence levels.

Product Description

The **DSQ Alert *Cryptococcus* species 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 DSQ.

Table 1. DSQ Alert *Cryptococcus* species 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)
<i>Cryptococcus neoformans</i> cytb gene	FAM	FAM
<i>Cryptococcus gattii</i> cytb gene	AP593	ROX, Texas Red
Internal control IC1	AP525	VIC, JOE, HEX

The **DSQ Alert Cryptococcus species 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 DSQ Alert Cryptococcus species RUO Detection Reagent.

Material	Use	Manufacturer	Part Number
MGB Alert® ELITaq Master Mix (2X)	Contains DNA polymerase, buffers, dNTPs, components for PCR	ELITechGroup	M800809, 48 reactions M800810, 480 reactions
Internal Control IC1 DNA	Internal control DNA template to monitor nucleic acid extraction and PCR performance	ELITechGroup	M800735
Molecular biology grade water	Reaction mix preparation, negative controls	NA	NA
Positive controls	Positive control DNA for the pathogen target	NA	NA

Recommended Reaction Setup

For optimal performance, protect all reagents from light, store them 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 DSQ Alert Cryptococcus species 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
DSQ 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 (45 cycles)	Denaturation	95°C	1 sec
	Annealing/extension*	63°C	1 min

* Read fluorescence at the annealing/extension stage of PCR.

Data Analysis Guidelines

Analysis of results from the DSQ Alert *Cryptococcus* species RUO Detection Reagent should be performed for the PCR stage. Amplification of FAM identifies the *Cryptococcus neoformans* species and amplification of AP593 identifies the *Cryptococcus gattii* species. Amplification of the internal control AP525 signal indicates the PCR performed as expected. Amplification of the internal control AP525 signal may or may not be observed in samples that test positive for *Cryptococcus species* DNA but must be observed in samples that test negative for *Cryptococcus species* 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 $\leq -10^{\circ}\text{C}$ 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 DSQ Alert labeling

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