MGB Alert® Candida auris with fluconazole resistance RUO Detection Reagent

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

SPIN TUBES PRIOR TO OPENING

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REF **M400861**

≤ -10°C

Intended Use

The **MGB Alert Candida auris with fluconazole resistance RUO Detection Reagent** is intended for use in a nucleic acid amplification test, to detect the presence of mutations associated with fluconazole resistance in *C. auris* DNA in a nucleic acid sample. This product is intended for use with a real-time PCR system with appropriate optical specifications and melt curve analysis capability.

Assay Principle

The **MGB Alert Candida auris with fluconazole resistance RUO Detection Reagent** is a multiplex real-time PCR reagent designed with MGB Pleiades® hybridization probe chemistry to detect and distinguish **fluconazole resistance-associated mutations and wild type in *C. auris***. To use this product effectively, thermal cycler parameters must include PCR thermal cycling with 5-color fluorescence detection and a dissociation stage, or melt stage. (See Recommended Reaction Setup below.) The reagent contains two primer sets and four probes specific to four mutations or the wild type genotype (Table 1), which are detected and distinguished by PCR and melt analysis. The reagent also contains a primer set and probe for an internal control (IC).

The fluorescence of unhybridized MGB Pleiades probes is quenched by a 5' minor groove binder (MGB) and a 3' Eclipse® Dark Quencher (EDQ). During each cycle of PCR, the primers and probes anneal to their target template, if present, and a new DNA strand is synthesized from the primers by a polymerase. Hybridization of the Pleiades probe to its target separates the fluorophore from its quencher and MGB, allowing fluorescence emission. As the polymerase encounters the probe annealed to the template downstream of the primer, the probe is displaced, and the quencher and MGB once again block fluorescence emission from the fluorophore. (An MGB on the 5' end of the Pleiades probe blocks the 5'-exonuclease activity of the polymerase, so a Pleiades probe is not hydrolyzed during PCR.) The PCR cycles result in exponential amplification of target DNA and fluorescence levels. The dissociation stage results in exponential decrease of fluorescence of the probe fluorophore, i.e., a melt curve. Amplification and the melt temperatures (T_ms) of the melt curves discriminate mutations and the wild type genotypes detected by each fluorophore.

Product Description

The **MGB Alert Candida auris with fluconazole resistance RUO Detection Reagent** is a ready-to-use 20X mix of primer and probe sets specific to the DNA of the target pathogen, and to a synthetic sequence that serves as an internal control to monitor assay performance. (The IC DNA template is sold separately, see below.) Probes are labeled with FAM or an AquaPhluor® (AP) fluorophore (Table 1), and an MGB and EDQ.

Table 1. MGB Alert Candida auris with fluconazole resistance RUO Detection Reagent components description. The number in the AP fluorophore name indicates its peak excitation wavelength.

Target template	Probe fluorophore	Analogous fluorophore (for optical channel selection)
C.auris ERG11 F126L mutation(s)	FAM	FAM
C.auris ERG11 Y132F mutation(s)	AP525	VIC, JOE, HEX
C.auris ERG11 K143R mutation(s)	AP593	ROX, Texas Red
C. auris TAC1b gene WT genotype	AP639	Cy5, Quasar 670
Internal control IC1	AP680	Cy5.5

The **MGB Alert Candida auris with fluconazole resistance RUO Detection Reagent** is provided at a volume of 120 µL, and 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 Candida auris with fluconazole resistance RUO Detection Reagent.

Material	Use	Vendor	Part Number
Internal Control IC1 DNA	Internal control DNA template to monitor nucleic acid extraction and PCR performance	ELITechGroup	M800735
MGB Alert® ELITaq Master Mix (2X)	Contains DNA polymerase with exonuclease activity, buffers, dNTPs, excipients 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 target genotype if available	NA	NA

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 Candida auris with fluconazole resistance 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	25.0
Molecular biology grade water	--	12.5
MGB Alert RUO Mix	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.
2. Array 40 µL of the reaction mix into the wells of an optical plate or tubes.
3. Prepare positive and negative controls and samples 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/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 and dissociation 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	10 sec
	Annealing*	56°C	30 sec
	Extension	72°C	30 sec
Dissociation (melt)	Hold	95°C	15 sec
	Annealing	40°C	15 sec
	Melt*	40→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 Candida auris with fluconazole resistance RUO Detection Reagent should be performed for both the PCR stage and dissociation stage. Amplification of FAM, AP525, and AP593 fluorescence signals during PCR indicates the sample is positive for *C. auris* DNA. Amplification of AP639 also indicates *C. auris* DNA is present, however, AP639 is indicative of a wild type genotype only. Absence of amplification of AP639 indicates either *C. auris* DNA is not present or a deletion in the TAC1b gene is present, signifying a fluconazole resistance genotype. (See Table 1.) Amplification of the internal control AP680 signal indicates the PCR performed as expected. Amplification of the internal control AP680 signal may or may not be observed in samples that test positive for *C. auris* DNA, but must be observed in samples that test negative for *C. auris* DNA to ensure the PCR performed as expected.

For the dissociation stage, each fluorescence signal that distinguishes a wild type from a fluconazole resistant genotype (FAM, AP525, or AP593) that amplified during PCR should be analyzed. The T_m (or T_ms) of each melt curve distinguish between the wild type and resistant genotype. More than one resistance genotype may be present in a nucleic acid sample. The absence of a resistance genotype does not preclude fluconazole resistance.

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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8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, 11155713, and 11320376 as well as applications that are currently pending.

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Symbols

The following symbols are used within ELITechGroup MDx MGB Alert® labeling

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