MGB Alert® M. genitalium with macrolide resistance RUO Detection Reagent

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

SPIN TUBES PRIOR TO OPENING

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REF **M400860**

≤ -10°C

Intended Use

The **MGB Alert® M. genitalium with macrolide resistance RUO Detection Reagent** is intended for use in a nucleic acid amplification test, to detect DNA from *Mycoplasma genitalium* and mutations associated with macrolide resistance 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 M. genitalium with macrolide resistance RUO Detection Reagent** is a multiplex real-time PCR reagent designed with both hydrolysis and hybridization probe chemistries to detect ***Mycoplasma genitalium* DNA** and distinguish **macrolide resistance-associated mutations and wild type in *M. genitalium***. To use this product effectively, thermal cycler parameters must include PCR thermal cycling and a dissociation stage, or melt stage. (See Recommended Reaction Setup below.) For the pathogen target, the reagent contains a single primer set and two probes each labeled with a fluorophore, a minor groove binder (MGB), and a quencher. The MGB hydrolysis probe is specific to *M. genitalium*, and serves to identify the species DNA by hydrolysis of this probe during PCR. The MGB Pleiades® hybridization probe serves to identify and distinguish a wild type genotype and several known resistance-conferring point mutations by melt curve analysis. The reagent also contains an internal control (IC) primer set and a hydrolysis probe labeled with a fluorophore and a duplex stabilizing quencher (DSQ).

The hydrolysis and hybridization probe chemistries in this product are unique. The fluorescence of unhybridized probes is quenched by an MGB and an Eclipse® Dark Quencher (EDQ), or a DSQ, which serves as an MGB and quencher in one molecule. 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. For the hydrolysis probes, during DNA synthesis the polymerase encounters the probe annealed to the template downstream of the primer, and the 5'-exonuclease activity of the polymerase hydrolyzes the probe, releasing the fluorophore from the proximity of its quencher and allowing fluorescence emission. For the Pleiades probe, hybridization of the probe to its target separates the fluorophore from its quencher and MGB, allowing fluorescence emission. As the polymerase encounters the Pleiades probe annealed to the template, the MGB on the 5' end of the probe blocks the exonuclease activity of the polymerase and the Pleiades probe is displaced. The PCR cycles result in exponential amplification of target DNA and fluorescence levels. The dissociation stage results in exponential decrease of fluorescence of the Pleiades probe fluorophore, i.e., a melt curve. The melt temperature (T_m) of the melt curve distinguishes wild type and the genotypes conferring macrolide resistance.

Product Description

The **MGB Alert M. genitalium with macrolide 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 either an MGB and EDQ, or a DSQ.

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

Target template	Probe type and chemistry	Probe fluorophore	Analogous fluorophore (for optical channel selection)	Additional probe labels
M. genitalium 23S rRNA gene species-specific region	MGB hydrolysis	AP525	VIC, JOE, HEX	MGB, EDQ
M. genitalium 23S rRNA wild type, or point mutations A2058G, A2059G, A2058T, A2058C, or A2059C	MGB Pleiades hybridization	FAM	FAM	MGB, EDQ
Internal control IC2	DSQ hydrolysis	AP639	Cy5, Quasar 670	DSQ

The **MGB Alert M. genitalium with macrolide resistance RUO Detection Reagent** is provided at a volume of 120 µL, and designed to be combined with a master mix containing the necessary excipients 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 M. genitalium with macrolide resistance RUO Detection Reagent.

Material	Use	Vendor	Part Number
Internal Control IC2 DNA	Internal control DNA template to monitor nucleic acid extraction and PCR performance	ELITechGroup	M800737
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 M. genitalium with macrolide 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
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.
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/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
UNG activation*	Hold	50°C	10 min
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 (melt)	Hold	95°C	15 sec
	Annealing	45°C	15 sec
	Melt**	40→80°C	Ramp at 0.06°C/sec

* The UNG activation step is optional and recommended when using a PCR master mix with UNG.
** Read fluorescence at the extension stage of PCR and while ramping during the melt stage of dissociation.

Data Analysis Guidelines

Analysis of results from the MGB Alert M. genitalium with macrolide resistance RUO Detection Reagent should be performed for both the PCR stage and dissociation stage. Amplification of the AP525 fluorescence signal during PCR is indicative of the presence of *M. genitalium* DNA in the nucleic acid sample. Amplification of the internal control AP639 signal indicates the PCR performed as expected. Amplification of the internal control

AP639 signal may or may not be observed in samples that test positive for *M. genitalium* DNA, but must be observed in samples that test negative for *M. genitalium* DNA to ensure the PCR performed as expected. Analysis of the amplification of the FAM signal during PCR is optional.

For the dissociation stage, only the FAM signal should be analyzed for each sample that tested positive for *M. genitalium* DNA. Mutations in the *M. genitalium* 23S gene are indicated by a sample T_m several degrees lower than the T_m of the wild type genotype.

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

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		