DSQ Alert™ Mycoplasma/Ureaplasma v2.0 RUO Monoreagent

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

SPIN TUBES PRIOR TO OPENING

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REF **M400791**

≤ -10°C

Intended Use

The **DSQ Alert Mycoplasma/Ureaplasma v2.0 RUO Monoreagent** is intended for use in a nucleic acid amplification test, to detect and distinguish DNA from *Mycoplasma* and *Ureaplasma* species 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 **DSQ Alert Mycoplasma/Ureaplasma v2.0 RUO Monoreagent** is a multiplex real-time PCR reagent designed with DSQ hydrolysis probe chemistry, the next generation of MGB hydrolysis probes, to detect and distinguish DNA from ***Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealiticum and Ureaplasma parvum species.*** To use this product effectively, thermal cycler parameters must include PCR thermal cycling with five color fluorescence detection. The reagent contains primers and a probe specific to the GAPDH gene for *Mycoplasma* species, and the urease gene for *Ureaplasma* 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 hydrolysis 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 Mycoplasma/Ureaplasma v2.0 RUO Monoreagent** is a ready-to-use mix of primer and probe sets specific to the DNA of the target pathogens, 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 a DSQ.

Table 1. DSQ Alert Mycoplasma/Ureaplasma v2.0 RUO Monoreagent 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 GAPDH gene species-specific region	DSQ hydrolysis	FAM	FAM	DSQ
M. hominis GAPDH gene species-specific region	DSQ hydrolysis	AP530	VIC, JOE, HEX	DSQ
U. parvum urease gene species-specific region	DSQ hydrolysis	AP593	ROX, Texas Red	DSQ
U. urealiticum urease gene species-specific region	DSQ hydrolysis	AP639	Cy5, Quasar 670, Alexa Fluor 647	DSQ
Internal control IC1	DSQ hydrolysis	AP690	Cy5.5, Quasar 705	DSQ

DSQ Alert Mycoplasma/Ureaplasma v2.0 RUO Monoreagent is provided at a volume of 1,000 µL per tube, 1 tube per kit, and contains all the necessary components for PCR except the template. The 1.250X monoreagent 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 Mycoplasma/Ureaplasma v2.0 RUO Monoreagent.

Material	Use	Vendor	Part Number
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 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 DSQ Alert Mycoplasma/Ureaplasma v2.0 RUO Monoreagent 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)
DSQ Alert RUO Monoreagent	1.250X	40.0
Sample/control template	--	10.0

1. Prepare reaction mix as above (Table 3) or adjust volumes per reaction based on 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 monoreagent 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
Polymerase activation	Hold	95°C	5 min
PCR (45 cycles)	Denaturation	95°C	10 sec
	Annealing/Extension**	63°C	25 sec

* The UNG activation step is optional and recommended when using a PCR master mix with UNG.
** Read fluorescence at the annealing/extension stage of PCR.

Data Analysis Guidelines

Analysis of results from the DSQ Alert Mycoplasma/Ureaplasma v2.0 RUO Monoreagent should be performed from the PCR stage. Amplification of the FAM fluorescence signal during PCR is indicative of the presence of *M. genitalium* DNA in the nucleic acid sample. Amplification of the AP530 fluorescence signal during PCR is indicative of the presence of *M. hominis* DNA in the nucleic acid sample. Amplification of the AP593 fluorescence signal during PCR is indicative of the presence of *U. parvum* DNA in the nucleic acid sample. Amplification of the AP639 fluorescence signal during PCR is indicative of the presence of *U. urealiticum* DNA in the 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 *Mycoplasma/Ureaplasma* DNA but must be observed in samples that test negative for *Mycoplasma/Ureaplasma* 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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DSQ Alert detection reagent are covered by one or more of U.S. Patents Numbers 6972339, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and 11320376 as well as applications that are currently pending.

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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
 N	Contains sufficient for <N> tests		