
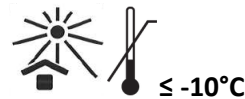


DSQ Alert™ MPXV/OPXV RUO Detection Reagent
For Research Use Only. Not for use in diagnostic procedures.

**SPIN TUBES
PRIOR TO
OPENING**

 ELITechGroup MDx LLC
21720 23rd Dr SE, Suite 150
Bothell, WA 98021 USA
Telephone: 1-800-453-2725

REF M400853



Intended Use

The **DSQ Alert™ MPXV/OPXV RUO Detection Reagent** is intended for use in a nucleic acid amplification test in a research laboratory setting, to detect and distinguish DNA from orthopox virus and monkeypox virus 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 MPXV/OPXV RUO Detection Reagent** is a multiplex real-time PCR reagent designed with DSQ hydrolysis probe chemistry, the next generation of MGB hydrolysis probes, to detect and distinguish **monkeypox virus (MPXV) and orthopox virus (OPXV) DNA**. For each target in the multiplex, the reagent contains a primer set and probe, labeled with a fluorophore and a **duplex stabilizing quencher (DSQ)**, to generate a fluorescent signal during PCR. 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 MPXV/OPXV RUO Detection Reagent** is a ready-to-use 20X mix of primer and probe sets specific to the DNA of each of the target pathogens, and to a synthetic sequence that serves as an internal control (IC) 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 that serves as a combined fluorescence quencher and DNA double helix stabilizer.

Table 1. DSQ Alert MPXV/OPXV RUO Detection Reagent components description. Each number in the AP fluorophore name indicates its peak excitation wavelength.

Target template	DSQ probe fluorophore	Analogous fluorophore (for optical channel selection)
OPXV DNA polymerase gene	AP593	ROX, Texas Red
MPXV TNF-binding protein J2R gene	FAM	FAM
Internal control IC2	AP525	VIC, JOE, HEX

The **DSQ Alert MPXV/OPXV 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.

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

Recommended Materials Not Provided

Table 2. Additional materials recommended for real-time PCR not provided in the DSQ Alert MPXV/OPXV RUO Detection Reagent.

Material	Use	Manufacturer	Part Number
Internal Control IC2 DNA	Internal control DNA template to monitor nucleic acid extraction and PCR performance	ELITechGroup	M800737
PCR master mix	Contains DNA polymerase with exonuclease activity, buffers, dNTPs, excipients for PCR	NA	NA
Molecular biology grade water	Reaction mix preparation, negative controls	NA	NA
Positive controls	Positive control DNA for each pathogen target primer/probe set	NA	NA

Recommended Reaction Setup

The following are examples of how to set up a real-time PCR using the DSQ Alert MPXV/OPXV RUO Detection Reagent for 25 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 recipes for real-time PCR reaction mix.

Reagent	Example 1 with 2X PCR master mix		Example 2 with 5X PCR master mix	
	Stock concentration	Volume per reaction (µL)	Stock concentration	Volume per reaction (µL)
PCR master mix	2X	12.50	5X	5.0
Molecular biology grade water	--	6.25	--	13.75
DSQ Alert RUO Detection Reagent	20X	1.25	20X	1.25
Total reaction mix	--	20.0	--	20.0
Sample/control template	--	5.0	--	5.0

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. Prepare positive and negative controls as appropriate.
3. Pipette 5 µL of sample or control into the appropriate well or PCR tube containing reaction mix.

4. Seal the plate with optical adhesive film or cap PCR tubes.
5. 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	5 min
PCR (45 cycles)	Denaturation	95°C	10 sec
	Annealing and extension**	60°C	30 sec

* The UNG activation step is optional and recommended when using a PCR master mix with UNG.

** Read fluorescence at the end of this cycle.

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 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.

Legal Notices

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Symbols

The following symbols are used within ELITechGroup MDx DSQ Alert labeling

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