

Protocol for Real-Time RT-PCR assay with Lyoph P&P Pan-Orthopox E9L (adapted to TaqMan platform)

For RUO (Research Use Only)

Vial content

Lyoph P&P Pan-Orthopox E9L contains lyophilized primers and probe (P&P) for detection of Orthopoxvirus (OPXV). It must be used in combination with a generic RT-qPCR kit.

Design

RT-qPCR systems included in Lyoph P&P Pan-Orthopox E9L :

- Smallpox and pan-Orthopox Virus Detection by Real-Time 3-Minor Groove Binder TaqMan Assays on the Roche LightCycler and the Cepheid Smart Cycler Platforms. Kulesh et al., Journal of clinical microbiology, 2004. [DOI: 10.1128/JCM.42.2.601-609.2004](https://doi.org/10.1128/JCM.42.2.601-609.2004)

Caution

Vials containing primers and probe mix (Lyoph P&P) must be stored at -20°C in the dark after reception. Stable 4 years under the described conditions.

Instructions

This protocol is adapted to the following kit and RT-qPCR platform:

- GoTaq® qPCR Master Mix (Promega)
- CFX (BIORAD) RT-qPCR thermal cycler

Use of other kits or RT-qPCR platforms may require adaptation of the protocol.

I. Rehydration of Lyoph-P&P

- Write the date on the vial before opening
- Resuspend Lyoph P&P as follows (**these steps are critical to ensure adequate homogenization**):
 - ✓ Add nuclease-free water or RE buffer as described in Table 1
 - ✓ Homogenize by pipetting 10 to 20 times the volume up and down in the glass vial
 - ✓ Incubate rehydrated P&P at room temperature for 10 min
 - ✓ Perform a second series of 10-times pipetting.

Table 1. Lyoph-P&P regeneration; the number of tests/vial is indicated on the Lyoph P&P vial label.

Packaging (number of tests/vial)	8+1	16+2	24+2	32+3	48+3	96+5
H ₂ O or RE buffer (µL)	63	126	182	245	357	707

II. Preparation of the PCR reaction mix (for GoTaq® qPCR Master Mix - Promega)

Table 2. Preparation of the PCR mix. *Rehydration performed as indicated in Table 1.

	Volume/PCR test (µL)
2X Master mix	13
Rehydrated Lyoph P&P*	7
Total (1 PCR test)	20
Template DNA (H ₂ O for Ctrl-)	5
Final volume	25

III. Cycling program and RT-qPCR (for GoTaq® qPCR Master Mix - Promega)

95°C	2min	45 cycles
95°C	15sec	
60°C	45sec (plate read)	

Read: FAM-QSY

Rehydrated Lyoph-P&P can be aliquoted and stored at -20°C. Each aliquot can be thawed only once and then discarded.