

# Protocol for Real-Time RT-PCR pan-Orthopoxviruses with P&P

**For RUO (Research Use Only)**

**Caution:** The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-MGB

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- GoTaq® Probe qPCR Mastermix kit, Promega ref A6101
- Water molecular grade

Nom	Number of vials	Number of assays/vial
Lyoph-P&P pan-Orthopox « OPE9L »	5	9

## 1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening.
- Lyoph-P&P is resuspended as described:
  - Add 63 µl of Water molecular grade
  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 30µL-volume
  - Rehydrated P&P must be incubated at room temperature for 10 min after which
- A second step of 10 times multiple pipetting must be done.

**WARNING:** These steps are critical to ensure adequate homogenization

**Table 1. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days**

Number of tests/vial	9
H2O (µL)*	63

**\* this volume is adapted to the GoTaq® Probe qPCR Mastermix kit and can vary depending upon the qPCR kit**

## 2. Preparation of the reaction MIX

MasterMix	25 $\mu$ L Single rxn, $\mu$ L
2X Reaction mix*	12.5
Rehydrated Primers and probe P&P**	7.0
H2O	0.5
<hr/>	
	20
Template DNA	5

\*, GoTaq® Probe qPCR Mastermix kit, Promega ref A6101

\*\* , as indicated in Table 2.

## 3. Cycling program and RT-PCR reaction

1: 95°C for 2 min

2: 95°C for 15 sec

3: 60°C for 45 sec

Plate Read

5: GOTO 2, 44 more times

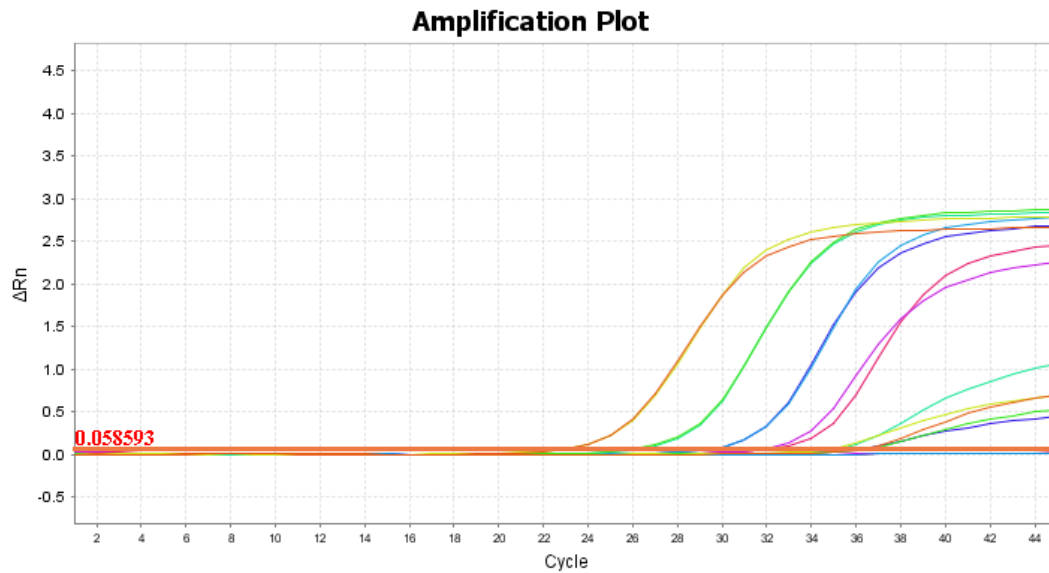


Figure. Results obtained with 10-fold serial dilutions of positive control for Monkeypox virus and Orthopoxviruses DNA (synthetic target-specific DNA) on QS12K Flex instrument .