

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

For RUO (Research Use Only)

Vial content

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

Systems : SYBR Green

Design

RT-qPCR system included in Lyoph P&P Pan-Phlebo Matsuno :

- Comprehensive Molecular Detection of Tick-Borne Phleboviruses Leads to the Retrospective Identification of Taxonomically Unassigned Bunyaviruses and the Discovery of a Novel Member of the Genus Phlebovirus. Matsuno et al., Journal of Virology, 2015. DOI: 10.1128/JVI.02704-14

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:

- QuantiNova SYBR Green RT-PCR kit (2500) QIAGEN
- 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)	43.2	86.4	124.8	168	244.8	484.8

II. Preparation of the PCR reaction mix (for QuantiNova SYBR Green RT-PCR kit QIAGEN)

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

	Volume/PCR test (μL)
2X Reaction mix	10
Rehydrated Lyoph P&P*	4.8
QN SYBR Green RT-Mix	0.2
Total (1 PCR test)	15
Template RNA (H ₂ O for Ctrl-)	5
Final volume	20

III. Cycling program and RT-qPCR (QuantiNova SYBR Green RT-PCR kit QIAGEN)

50°C	10min	45 cycles
95°C	2min	
95°C	10sec	
50°C	20sec	
72°C	40sec (plate read)	
95°C	1min	

Melt curve 60°C to 94°C, increment 0.5°C for 5 sec

Read: SYBR Green

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