

Protocol for Real-Time RT-PCR assay with Lyoph P&P TOSV Trio (adapted to TaqMan platform)

For RUO (Research Use Only)

Vial content

Lyoph P&P TOSV Trio contains lyophilized primers and probe (P&P) for detection of Toscana virus (TOSV). It must be used in combination with a generic RT-qPCR kit.

Probe: FAM-QSY

Design

RT-qPCR systems included in Lyoph P&P TOSV Trio:

- Pérez-Ruiz M, Collao X, Navarro-Marí JM, Tenorio A. Reverse transcription, real-time PCR assay for detection of Toscana virus. J Clin Virol. 2007 Aug;39(4):276-81. DOI: 10.1016/j.jcv.2007.05.003.
- Weidmann M, Sanchez-Seco MP, Sall AA, Ly PO, Thiongane Y, Lô MM, Schley H, Hufert FT. Rapid detection of important human pathogenic Phleboviruses. J Clin Virol. 2008 Feb;41(2):138-42. DOI: 10.1016/j.jcv.2007.10.001.
- Brisbarre N, Plumet S, Cotteaux-Lautard C, Emonet SF, Pages F, Leparç-Goffart I. A rapid and specific real time RT-PCR assay for diagnosis of Toscana virus infection. J Clin Virol. 2015 May;66:107-11. DOI: 10.1016/j.jcv.2015.03.007

Evaluation of Trio TOSV assay:

- Thirion L, Pezzi L, Pedrosa-Corral I, Sanbonmatsu-Gamez S, de Lamballerie X, Falchi A, Perez-Ruiz M, Charrel RN. Evaluation of a Trio Toscana Virus Real-Time RT-PCR Assay Targeting Three Genomic Regions within Nucleoprotein Gene. Pathogens. 2021 Feb 24;10(3):254. DOI: 10.3390/pathogens10030254.

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:

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher)
- 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 SuperScript™ III Platinum™ One-Step qRT-PCR Kit - ThermoFisher)

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

	Volume/PCR test (μL)
2X Reaction mix	12.5
Rehydrated Lyoph P&P*	7
SSIII/Taq enzyme mix	0.5
Total (1 PCR test)	20
Template RNA (H ₂ O for Ctrl-)	5
Final volume	25

Read: FAM-QSY

III. Cycling program and RT-qPCR (for SuperScript™ III Platinum™ One-Step qRT-PCR Kit - ThermoFisher)

50°C	15min	45 cycles
95°C	2min	
95°C	15sec	
60°C	45sec (plate read)	

Read: FAM-QSY

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