Protocol for Real-Time RT-PCR assay with Lyoph P&P DENV all Duo (adapted to TaqMan platform)

For RUO (Research Use Only)

Vial content

Lyoph P&P DENV all Duo contains lyophilized primers and probe (P&P) for detection of pan-dengue virus (DENV), with no serotyping. It must be used in combination with a generic RT-qPCR kit.

NB: Probe: FAM-QSY

Design

RT-qPCR systems included in Lyoph P&P DENV all Duo:

- Early diagnosis of dengue in travelers: Comparison of a novel real-time RT-PCR, NS1 antigen detection and serology. Huhtamo et al., J Clin Virol, 2010. DOI: 10.1016/j.jcv.2009.11.001
- Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses. Leparc-Goffart et al., J Clin Virol, 2010. DOI : 10.1016/j.jcv.2009.02.010

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_2O 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_2O for Ctrl-)	5
Final volume	**25**

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

50°C	15min	
95°C	2min	
95°C	15sec	45 cycles
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.

Caution: The DENV Duo assay amplifies a region of the 3'UTR that is well conserved between the 4 serotypes and between Flaviviruses. In case of very high viral load with Zika virus (Ct values <25, very rarely observed), it is possible to have false positives results with the DENV Duo system (generally Ct>36).