

Protocol for the use of a vial of Inactivated Virus as positive control arbovirus

Vial Content

An inactivated virus vial contains non-infectious, lyophilized material: inactivated virus spiked in plasma. This vial can be extracted and tested using the usual molecular diagnostic protocol.

Caution

Vials of inactivated virus must be stored at -20°C away from light after receipt. Stable for at least 6 months under the conditions described.

Instructions

This protocol is adapted to the following kit and extraction/RT-qPCR platforms:

- EZ1/EZ2 Virus Mini Kit v2.1 (QIAGEN) on EZ1/EZ2 (QIAGEN)
- SuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher)
- Thermocycler CFX (BIORAD)

This vial of inactivated virus cannot be used as a specificity control. It is not guaranteed to be free of other viral RNAs.

I. Rehydration of the lyophilizate

- Write the date on the bottle before opening it.
- Resuspend the lyophilizate as follows: **(these steps are essential to ensure adequate homogenization)**
 - ✓ Add 400µL of distilled water to the vial.
 - ✓ Pipette the volume up and down in the vial 10 to 20 times to ensure adequate homogenization
 - ✓ Leave the rehydrated lyophilizate at room temperature for 10 minutes
 - ✓ Perform a second pipetting step after incubation

This production batch has been validated according to the following protocol. However, the use of other kits or other extraction or RT-qPCR instruments may also be considered.

II. Preparation of the working solution

- Extract from 400 µL of the stock solution using the EZ1/EZ2 automated system (QIAGEN) with EZ1/EZ2 Virus Mini Kit v2.1
- Select 60 µL as the elution volume
- If the positive control is used several days after extraction, the working solution must be aliquoted to avoid multiple freeze/thaw cycles and stored at -80°C for optimal stability.

III. RT-qPCR

- Prepare PCR mix using RT-qPCR assay Lyoph-P&P (lyophilized primers and probe), previously rehydrated as explained in the SoP 'Protocol for Real-Time RT-PCR assay with Lyoph-P&P (adapted to TaqMan platform)' (Table 1)
- Perform RT-qPCR with the cycling program in Table 2

Table 1. Preparation of the RT-qPCR mix (using SuperScript™ III Platinum™ One-Step qRT-PCR Kit - ThermoFisher).

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

Table 2. Cycling program.

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

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