

# Protocol for Real-Time RT-PCR assay with Lyoph P&P Lassa 2018 (adapted to TaqMan platform)

For RUO (Research Use Only)

## Vial content

Lyoph P&P Lassa 2018 contains lyophilized primers and probe (P&P) for detection of Lassa virus (LASV). It must be used in combination with a generic RT-qPCR kit.

Probe: FAM-QSY

## Design

RT-qPCR system included in Lyoph P&P Lassa 2018 :

- International External Quality Assessment Study for Molecular Detection of Lassa Virus. Nikisins et al., PLOS Neglected Tropical Diseases, 2015. DOI: 10.1371/journal.pntd.0003793

## 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 <sub>2</sub> 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                  |
| <b>Total (1 PCR test)</b>                 | <b>20</b>            |
| Template RNA (H <sub>2</sub> O for Ctrl-) | 5                    |
| <b>Final volume</b>                       | <b>25</b>            |

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              |           |
| 55°C | 45sec (plate read) |           |
| 72°C | 15sec              |           |

Read: FAM-QSY

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