

Protocol for Real-Time RT-PCR of EboZaire DUO with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Development and evaluation of a duo Zaire ebolavirus Real-Time RT-PCR assay targeting two regions within the genome. Thirion L, Charrel R. N., Boehmann Y., Corcostegui I., Raoul H., Lamballerie X. *Microorganisms*, 2019, 7 (12), art. 652 [15 p.]

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088
- Water molecular grade
- IVT RNA Ebola Z specific

1. Kit contents :

| Nom | Number of vials | Number of assays/vial |
|------------------------------|-----------------|-----------------------|
| QC_Lyoph-P&P EboZaire Duo | | 8+1 |

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is resuspended as described:
 - Add 63 µl of Water molecular grade
 - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 30µL-volume
 - Rehydrated P&P must be incubated at room temperature for 10 min after which
 - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

| | |
|----------------------|----|
| Number of tests/vial | 9 |
| H2O (µL) | 63 |

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. Preparation of the reaction mix

| MasterMix | 25µL Single rxn, µL |
|------------------------------------|------------------------|
| 2X Reaction mix* | 12,5 |
| Rehydrated Primers and probe P&P** | 7 |
| SSIII/Taq Enzyme Mix* | 0,5 |
| Total | 20 |
| Template RNA | 5 |

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

** , as indicated in Table 2.

3. Cycling program and RT-PCR reaction

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 30sec – Plate read

5 : GOTO3, 45 more time

| |
|------------------|
| IVT Ebola Z 10-9 |
| Mean Ct : 26.11 |

Result obtain with DuoEboZ specific assay and 5µL of positive control

