

Protocol for Real-Time RT-PCR Marburg 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-QSY

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
- Elution Buffer AE (1000 ml) Macherey-Nagel™ ref 740917.1 (This component is not provided with the OneStep RT-PCR kit); usually provided as elution buffer in the extraction kit, (10mM TrisHCl 0.5mM EDTA, pH=9).

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 182 µl of AE Elution buffer
- Homogenization by 10 to 20 times pipetting up and down in the glass vial a 90µL 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

Table 1. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 14 days

Number of tests/vial	24(+2)
AE Elution buffer (µL)	182*

*** 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

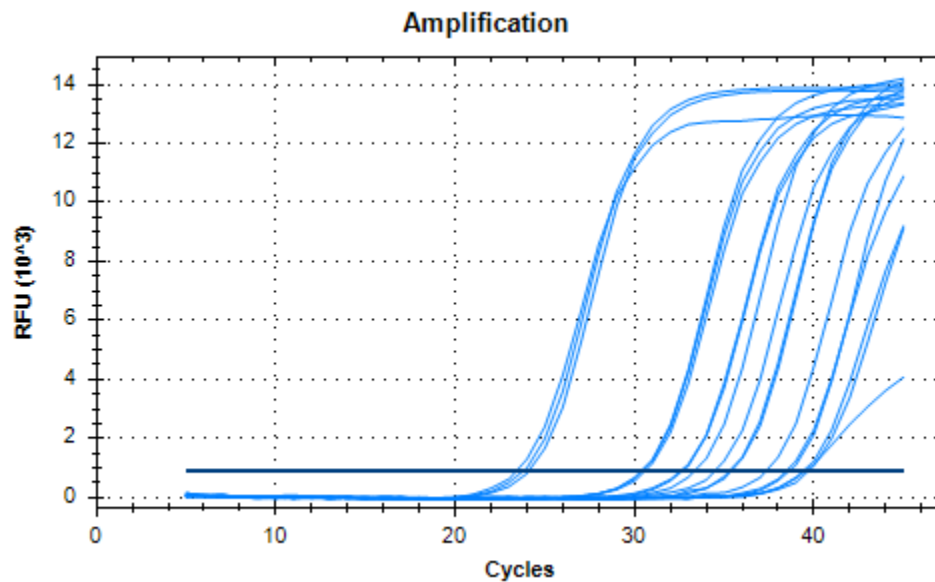
MasterMix	25 μ L Single rxn, μ L
2X Reaction mix*	12.5
Rehydrated Primers and probe P&P**	7.0
SSIII/Taq EnzymeMix*	0.5
	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase **, as indicated in Table 1.

3. Cycling program and RT-PCR reaction

- 1: 50°C for 15 min
 - 2: 95°C for 2 min
 - 3: 95°C for 15 sec
 - 4: 60°C for 45 sec
- Plate Read
- 5: GOTO 3, 44 more times

Figure. Results obtained with 10-fold serial dilutions of positive control in duplicate



LOD 95 <1 copy/ μ L