

**Protocol for Real-Time RT-PCR Severe Fever with Thrombocytopenia Syndrome virus 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 + VIC-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
- Water molecular grade
- Positive control SFTSV

**Contenu du kit :**

Nom	Number of assays/vial
QC_Lyoph-P&P SFTSV_N.SEG_YOSH	24
QC_Lyoph-P&P NotI	8
Positive Control – SFTSV	1000

**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 182 µl of Water molecular grade
  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 100µL-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	24	8
H2O (µL)	182	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** SFTSV-FAM	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: 95°C – 2min

2: 95°C for 15sec

3: 60°C for 45sec – Plate read

4: GOTO2, 45 more time

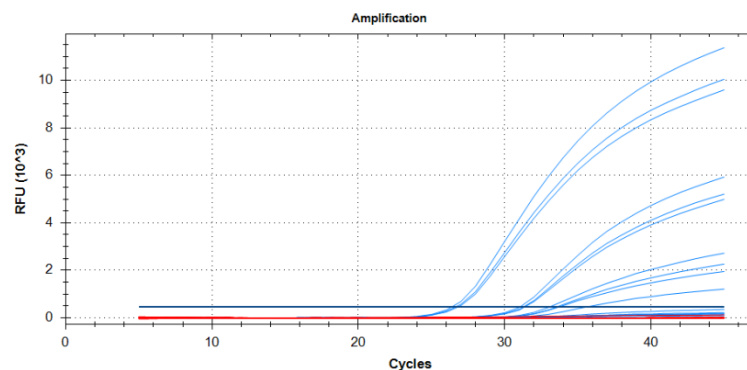


Figure obtained with QC\_Lyoph-P&P SFTSV\_N.SEG\_YOSH assay, 5µL of positive control tested.

## **4. Preparation of positive control SFTSV**

Resuspend the positive control in 500µL of water (molecular grade), make 50µL aliquotes of it and store at -20°C.

## **5. Contamination detection**

To detect if you have an DNA contamination make a mixture with both system SFTSV and NotI. NotI is a probe emitted in VIC channel and detect the positive control. If you're sample is positive for FAM (SFTSV) and VIC (Not1), the positive signal is due to positive control.

Make this assay for each positive sample you have. Make sure to resuspended each lyoph Pox and

MasterMix	25µL Single rxn, µL
2X Reaction mix	12,5
Rehydrated Primers and probe P&P** SFTSV - FAM	3,5
Rehydrated Primers and probe P&P** NotI - VIC	3,5
SSIII/Taq EnzyleMix	0,5
Total	20
Template RNA	5

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

\*\* , as indicated in Table 3.

Not1 at 2X to make a master mix with both tubes.

#### **Preparation of the reaction mix with NotI**

Number of tests/vial	24	8
H2O (µL)	91	31,5

**Table 3. 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**