

Protocol for Real-Time RT-PCR of SINV 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

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 Alpha

1. Kit contents :

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P Sindbis	20	24+2

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 100 µL-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	26
H2O (µL)	182

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 Alpha 10-8
Mean Ct : 29.82

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

