#### 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<br>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 respudended 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

<sup>\*</sup> 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<br>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                      |

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

## 3. Cycling program and RT-PCR reaction

1:50°C - 15min

2:95°C - 2min

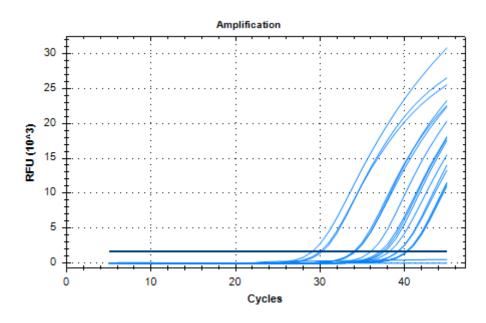
3:95°C-15sec

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  $5\mu L$  of positive control



<sup>\*\*,</sup> as indicated in Table 2.