# Protocol for Real-Time RT-PCR SARS-CoV-2 (2019-nCoV) (N Gene/CUB) 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 respudended as described:
  - Add 182 µl of AE Elution buffer
  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 50µL-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** |
|---|---|
| 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** | **25µL Single rxn, µL** |
|---|---|
| 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:** 58°C for 45 sec
Plate Read
**5:** GOTO 3, 44 more times

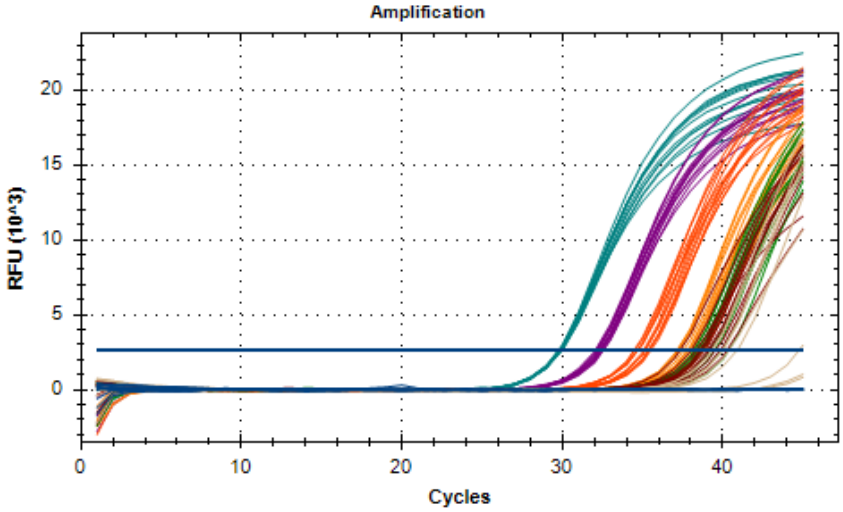


**Figure. Results obtained with 5-fold serial dilutions of positive control.**