

# Technical Description of the Armored RNA NTD + RBD BavPat1 (EU)

For RUO (Research Use Only)

**Caution:** The vials containing the Armored RNA (ArRNA) should be stored at -20°C in the dark. Stable at least 6 months in these conditions.

A vial contains material for 100 reactions.

The dilution of the ArRNA was adjusted to provide approximately  $4 \times 10^3$  copies of RNA/ $\mu$ L

## 1. Rehydration of Lyophilized ArRNA

- Write the date on the vial before opening.
- Resuspend the ArRNA in 500 $\mu$ L RNase free water / vial.
- Homogenize by pipetting up and down in the glass vial a 250 $\mu$ L-volume 10 to 20 times
- Rehydrated ArRNA must be incubated at room temperature for 5 min before spiking.
- The total preparation or only a fraction of the preparation can be used for spiking followed by the routine extraction protocol. The remaining volume can be aliquoted and stored at -20°C or -80°C.

## 2. Protocol used for validation of the ArRNA BavPat1 at UVE (Unité des Virus Emergents, Marseille, France)

- 500 $\mu$ L of RNase free was added in a vial and the ArRNA was resuspended as described above.
- 400 $\mu$ L were used for Extraction on EZ1 (Qiagen) platform using EZ1 Virus Mini Kit v2.1 (cat 955134)
- Nucleic acids were eluted in 60 $\mu$ L
- Nucleic acids were diluted in 440 $\mu$ L of PBS and 5 $\mu$ L was used for real time RT-PCR with qRT-PCR One-step SuperScript™ III Platinum™ kit, using 12.5 $\mu$ L of 2X Mix, 7 $\mu$ L of lyophilized Primers and Probes, 0.5 $\mu$ L of Reverse Transcriptase and 5 $\mu$ L of sample. The cycling conditions were defined as follow : 50°C for 15 min, 95°C for 2 min and 45 cycles of 95°C 15 sec; 60°C 45 sec.