Technical Description of the Armored RNA RdRp Wuhan/CUB

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 is developed for 25 reactions with expected Ct between 27-30 and contains 2,37E+06 RNA copies/vial

1. Rehydration of Lyophilized ArRNA

- Write the date on the vial before opening.

- Resuspend the ArRNA in 400µL RNase free water / vial.

-Homogenize by pipetting up and down in the glass vial a 50µL-volume 10 to 20 times

- Rehydrated ArRNA must be incubated at room temperature for 5 min before extraction

- The total preparation or only a fraction of the preparation can be used for extraction using the routine extraction protocol. The remaining volume can be stored at -20°C or -80°C.

- After extraction, the RNA can be used in the same conditions as for an *in vitro* transcript (used as positive control for the molecular assay, or aliquoted and stored at -80°C for optimal stability).

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

- 400 μL of RNAse free were added in a vial and the ArRNA was resuspended as described above.
-400μL were used for Extraction on EZ1 (Qiagen) platform using EZ1 Virus Mini Kit v2.1 (cat 955134).
- Nucleic acids were eluted in 60μl, EA buffer is added to a final volume of 125μL, and 5μl was used for RT-PCR following the previously described protocol https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf