Technical Description of the Armored RNA MS2 (1)

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.

This Armored RNA was validated in nasopharyngeal, salivary, urine and stool samples.

The dilution of ArRNA MS2 was adjusted to provide a Cycle threshold (Ct) at ~30 for real time PCR assays upon extraction and amplification of 200µL of **nasopharyngeal, salivary, urine and stool samples** spiked with the ArRNA MS2.

1. Rehydration of Lyophilized ArRNA

- Write the date on the vial before opening.
- Resuspend the ArRNA in 1mL RNase free water / vial.
- Homogenize by pipetting up and down in the glass vial a 250µL-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 MS2 at UVE (Unité des Virus Emergents, Marseille, France)

- 1mL of RNAse free was added in a vial and the ArRNA was resuspended as described above.
- 10µL of ArRNA were added to 200µL of the biological sample
- 200µL of VXL (Qiagen) were added and incubated at room temperature for 15 min
- 400µL were used for Extraction on EZ1 (Qiagen) platform using EZ1 Virus Mini Kit v2.1 (cat 955134)
- Nucleic acids were eluted in 60µl, and 5µl was used for real time RT-PCR with the following set of primers and probes: MS2 phage (MS2F CTCTGAGAGCGGCTCTATTGGT, MS2R GTTCCCTACAACGAGCCTAAATTC, MS2probe *VIC*-TCAGACACGCGGTCCGCTATAACGA-*TAMRA*) (https://www.european-virus-archive.com/detection-kit/lyophilized-primers-and-probe-rt-pcr-ms2-

phage-used-internal-control). Real time RT-PCR was performed with qRT-PCR One-step SuperScript™ III Platinium™ kit (thermofischer), using 12.5µL of 2XMix, 7µL of lyophilized Primers and Probes, 0.5µL of Reverse Transcriptase and 5µL of extracted RNA. The cycling conditions were defined as follows : 50°C for 15 min, 95°C for 2 min and 45 cycles of 95°C 15 sec; 60°C 45 sec.