

Technical Description of the Armored RNA WestNile DUO

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.

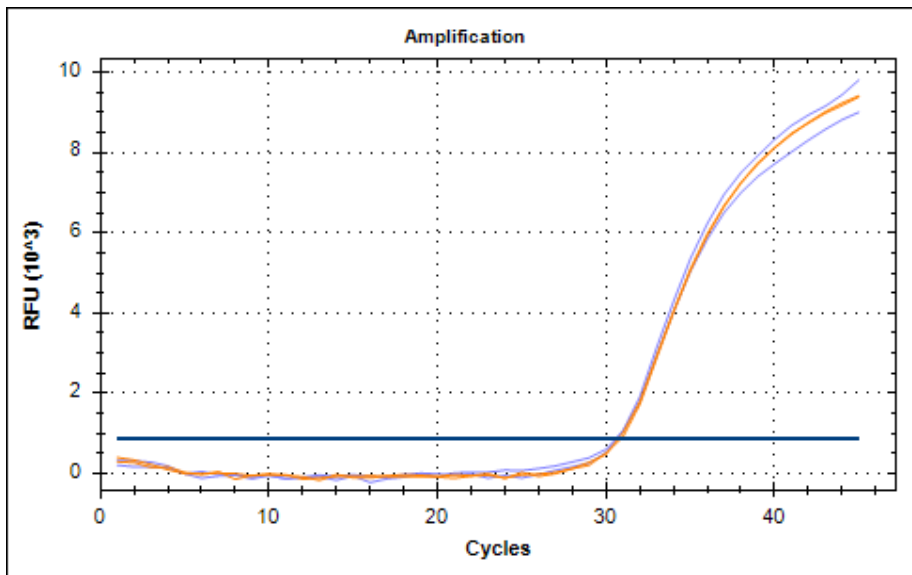
The dilution of the ArRNA was adjusted to provide approximatively an amplification of 30 CT

1. Rehydration of Lyophilized ArRNA

- Write the date on the vial before opening.
- Resuspend the ArRNA in 500µL 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 WestNile Duo at UVE (Unité des Virus Emergents, Marseille, France)

- 500µL of RNase free was 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
- Nucleic acids were diluted in 440µl of PBS and 5µl was used for real time RT-PCR with qRT-PCR One-step SuperScript™ III Platinum™ kit, using 12.5µL of 2X Mix, 7µL of lyophilized Primers and Probes, 0.5µL of Reverse Transcriptase and 5µL 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.



CT

Tube 1	30,60
	30,52
Tube 2	30,83
	30,67