

Real-time RT-PCR (TaqMan™) protocol – Yellow fever virus (YFV) 17D vaccine strain

1. Master mix

| Component | Volume per reaction | Volume for 10 reactions | Volume for 50 reactions |
|---------------------------|---------------------|-------------------------|-------------------------|
| RNase/DNase-free water | 1.35 µl * | 13.5 µl * | 67.5 µl * |
| reaction buffer (2x) | 12.5 µl * | 125 µl * | 625 µl * |
| forward primer (100 µM) | 0.25 µl | 2.5 µl | 12.5 µl |
| reverse primer (100 µM) | 0.25 µl | 2.5 µl | 12.5 µl |
| probe (25 µM) | 0.15 µl | 1.5 µl | 7.5 µl |
| enzyme | 0.5 µl * | 5 µl * | 25 µl * |
| Total per reaction | | 15 µl | |

2. RNA

Add **10 µl** of RNA to 15 µl of master mix.

Include positive and negative **controls** to evaluate the validity of the run.

3. Cycling conditions

1 cycle:

50°C for 30 min * (reverse transcription)

95°C for 2 min * (DNA polymerase activation, “hot start”)

45 PCR cycles:

95°C for 15 seconds

64°C for 1 min

4. Interpretation

assay **validity**: positive and negative controls should show the expected results

5. Primers and probes

Hughes HR et al., Development of a real-time reverse transcription-PCR Assay for global differentiation of yellow fever virus vaccine-related adverse events from natural infections. *J Clin Microbiol.* 2018;**56**(6)

forward primer

5'- GTATTCTGTGGATGCTGACC

reverse primer

5'- TATCCCGGTTTCAGGTTGTG

probe (R)

5'- FAM-CTC**ACCCAG**TTGCAG-BHQ-1

Bold type bases in the probe have locked nucleic acid (LNA) chemistry.

* The volumes and times indicated are for the use of the SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen, catalog number: 11732-020 or 11732-088) and should be adjusted when other enzymes are used.