

## Real-time RT-PCR (TaqMan™) protocol - Yellow fever virus (YFV)

### 1. Master mix

Component	Volume per reaction	Volume for 10 reactions	Volume for 50 reactions
RNase/DNase-free water	6.4 µl *	64 µl *	320 µl *
reaction buffer (2x)	12.5 µl *	125 µl *	625 µl *
primer 1 (100 µM)	0.25 µl	2.5 µl	12.5 µl
primer 2 (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 *
<b>Total per reaction</b>		<b>20 µl</b>	

### 2. RNA

Add **5 µl** of RNA to 20 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  
60°C for 1 min

### 4. Interpretation

**positivity:** Ct value ≤ 38  
assay **validity:** positive and negative controls should show the expected results

### 5. Primers and probes

The use of the following primers and TaqMan™ probes is recommended:

Domingo <i>et al.</i> , <i>J Clin Microbiol</i> <b>50</b> , 4054-60 (2012)	
YFallF (YF15-38F)	5'-GCTAATTGAGGTGYATTGGTCTGC
YFallP (YF41-64FAM)	5'-FAM-ATCGAGTTGCTAGGCAATAAACAC-BHQ1
YFallR (YF83-103R)	5'-CTGCTAATCGCTCAAMGAACG

or

Johnson <i>et al.</i> , CDC (not published)	
YF14-34F	5'-TGCTAATTGAGGTGCATTGG
YF34-57FAM	5'-FAM-TCTGCAAATCGAGTTGCTAGGCA-BHQ1
YF115C	5'-CTGGTCAGTTCTCTGCTAATCG

\* 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.