

Real-time RT-PCR (TaqMan™) protocol - Zika virus (ZIKV)

1. Master mix

Component	Volume per reaction	Volume for 10 reactions	Volume for 50 reactions
RNase/DNase-free water	1.4 µl *	14 µl *	70 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 *
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

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:

Lanciotti <i>et al.</i> , <i>Emerg Infect Dis</i> 14 , 1232-9 (2008) [†]	
ZIKV 1087	5'-CCGCTGCCCAACACAAG
ZIKV 1108FAM	5'-FAM-AGCCTACCTTGACAAGCAGTCAGACACTCAA-BHQ1
ZIKV 1163c	5'-CCACTAACGTTCTTTTGCAGACAT

or

Lanciotti <i>et al.</i> , CDC (not published, specific for ZIKV Asian genotype)	
ZIKV 4481	5'- CTGTGGCATGAACCCAATAG
ZIKV 4507cFAM	5'-FAM- CCACGCTCCAGCTGCAAAGG -BHQ1
ZIKV 4552c	5'-ATCCCATAGAGCACCCTCC

* 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.

[†] These primers and probes were originally called ZIKV 1086/1162c/1107FAM.

Disclaimer: The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the Pan American Health Organization in preference to others of a similar nature that are not mentioned.