

Real-time RT-PCR protocol - Mayaro (MAYV) and Oropouche virus (OROV) duplex

- Duplex assay for the detection of:
 - MAYV
 - S segment of OROV and OROV-like viruses
- Reference: Naveca et al., *Mem Inst Oswaldo Cruz*, Vol. 112(7): 510-513, July 2017

1. Master mix

Components	Volume per reaction	Volume per reaction
	SuperScript™ III Platinum™ One-Step qRT-PCR Kit ¹	AgPath-ID™ One-Step RT-PCR Reagents ²
RNase/DNase-free water ³	1.8 µl	1.4 µl
reaction buffer(2x) ³	10.0 µl	10.0 µl
MAYV_FNF primer (10 µM)	0.6 µl	0.6 µl
MAYV_FNR primer (10 µM)	0.6 µl	0.6 µl
MAYV_FNP probe (10 µM)	0.2 µl	0.2 µl
OROV_FNF primer (10 µM)	0.6 µl	0.6 µl
OROV_FNR primer (10 µM)	0.6 µl	0.6 µl
OROV_FNP probe (10 µM)	0.2 µl	0.2 µl
enzymes ³	0.4 µl	0.8 µl
Total per reaction	15 µl	

2. RNA

Add **5 µl** of RNA to 15 µl of master mix (total reaction volume: 20 µl).
Include positive and negative **controls** to evaluate the validity of the run.

3. Cycling conditions⁴

SuperScript™ III Platinum™ One-Step qRT-PCR Kit	AgPath-ID™ One-Step RT-PCR Reagents
Hold steps:	Hold steps:
50°C for 15 min (reverse transcription)	50°C for 15 min (reverse transcription)
95°C for 2 min (DNA polymerase activation)	95°C for 10 min (DNA polymerase activation)
45 PCR cycles:	45 PCR cycles:
95°C for 15 seconds	95°C for 15 seconds
60°C for 30 seconds (acquire fluorescence at this step)	60°C for 30 seconds (acquire fluorescence at this step)

For fluorescence acquisition:

- MAYV: set probe dye to VIC.
- OROV: set probe dye to FAM.

4. Interpretation

positivity: Ct value ≤ 38

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

¹ Invitrogen, cat. no.: 11732-020 or 11732-088.

² Applied Biosystems, cat. no.: 4387391 or 4387424.

³ The volumes indicated are for the use of the SuperScript™ III Platinum™ One-Step qRT-PCR Kit or AgPath-ID™ One-Step RT-PCR Reagents, and should be adjusted when other enzymes are used.

⁴ The duration of reverse transcription and DNA polymerase activation listed are for the SuperScript™ III Platinum™ One-Step qRT-PCR Kit or the AgPath-ID™ One-Step RT-PCR Reagents, and should be adjusted when other enzymes are used.

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.

5. Primers and probes

MAYV_FNF	5'-CACGGACMTTTTGCCTTCA
MAYV_FNR	5'-AGACTGCCACCTCTGCTKGAG
MAYV_FNP	5'-VIC-ACAGATCAGACATGCAGG-NFQ-MGB
OROV_FNF	5'-TCCGGAGGCAGCATATGTG
OROV_FNR	5'-ACAACACCAGCATTGAGCACTT
OROV_FNP	5'-FAM-CATTTGAAGCTAGATACGG-NFQ-MGB