

rtRT-PCR for the detection of O/EA-3 FMDV

This real-time RT-PCR is a molecular tool for detection of Foot-and-mouth disease virus topotype O/EA-3 (from West and North Africa). Topotype EA-3 of serotype O circulates particularly in Africa, with a dissemination from the East to the West, and then to North Africa. The assay has been tested on 34 samples from West and North Africa with a specificity of 94,1% (16/17 EA-3 samples detected). One cross-reaction with an old O/WA sample was observed. The primers and probe are indicated hereafter:

Oligo name (final concentration)	Sequence (5'-3')	Use
OEA3_F (0.4 µM)	AACGTCTTGGACCTGATGCAG	Forward Primer
OEA3_P (0.3 µM)	FAM-CACACGCTGGTTGGGGCGCTCCT-TAMRA	Probe
OEA3_R (0.4 µM)	CCCTCGTGCTTCACTGCTACTTC	Reverse Primer

The O/EA-3 rtRT-PCR assay has been validated using Ag-Path kit in a duplex system with β -actin, and following the volumes and concentrations as follow (5 µl of RNA):

	Volume (µl)	Concentration		
	For one tube	Initial	Final	
Ultrapure water (DNase RNase Free)	1,15	/		
Buffer 2X (kit AgPath-ID™)	12,5	2	1	X
Primer F	1	10	0,4	µM
Primer R	1	10	0,4	µM
Probe FAM-TAMRA	0,75	10	0,3	µM
Primer F β-actine	1	10	0,4	µM
Primer R β-actine	1	10	0,4	µM
Probe VIC-TAMRA β-actine	0,6	5	0,12	µM
RT-PCR mix 25X (Enzyme)	1	25	1	X

Oligo name (final concentration)	Sequence (5'-3')	Use
β -actine F (0.4 µM)	CAGCACAATGAAGATCAAGATCATC	Forward Primer
β -actine R (0.4 µM)	CGGACTCATCGTACTCCTGCTT	Reverse Primer
β -act P (0.3 µM)	VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA	Probe

Real-time PCR program:

Cycles of RTq-PCR		
T°	Time	nb cycles
45°C	10min	1
95°C	10min	1
95°C	15s	45
60°C	1min	

NB: This system has been validated on a small number of samples and should therefore be tested against other samples from this lineage.