rtRT-PCR for the detection of SAT2/V FMDV

This real-time RT-PCR is a molecular tool for detection of Foot-and-mouth disease virus topotype SAT2/V. In 2023, outbreaks of serotype SAT2/V were detected in North Africa. The assay developed is specifically targeting the topotype V of serotype SAT2. The assay has been tested on 65 samples with a specificity of 100% (36/36 for SAT2/V samples). The primers and probe are indicated hereafter:

Oligo name (final concentration)	Sequence (5'-3')	Use
SAT2_V_F (0.8 µM)	GCGGTTTTGGCGAGCAAATA	Forward Primer
SAT2_V_probe (0.3 µM)	FAM-AACAAGAAGCACACACTGCCGTCC-TAMRA	Probe
SAT2_V_R (0.8 µM)	ACGGTAGTAAACGTCGACTGG	Reverse Primer

The SAT2/V 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	20	0,8	µM
Primer R	1	20	0,8	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.