rtRT-PCR for the detection of SAT1/III (“BOT/1/77 like”)

This real-time RT-PCR assay is a molecular tool designed to detect Foot-and-Mouth Disease viruses belonging to the SAT1/III topotype, which have been circulating in the Middle East and the European Union since late 2025. These viruses are closely related to the BOT/1/77 isolate, which is used as a vaccine strain by some companies. At present, this assay is considered a candidate test and has not yet been fully validated; however, it may be useful for the early detection of this specific topotype.
The primers and probe are indicated hereafter:

Oligo name (final concentration)	Sequence (5’-3’)	Use	Position (VP1)
SAT1_III_2026_F (0.4 µM)	ACTCTGGTTGGGAAGACTACTG	Forward Primer	118-139
SAT1_III_2026_P (0.3 µM)	FAM-CATGGTTCTGGATCTTCTGAGCACCAA-TAMRA	Probe	147-173
SAT1_III_2026_R (0.4 µM)	GGCGCCGACCAGTGACTT	Reverse Primer	178-195

The SAT1/III 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-actine 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 topotype.