

# **MMS FMDV qPCR Detection Assay**

## **Product Manual**

### **Version 1.0**

This product is intended for research use only.

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## A. Introduction and Principles of the Test

This duplex, probe-based real-time qPCR assay is intended for detection of all known strains of the Foot-and-Mouth-Disease Virus (FMDV) in a little over 1 hour (with extracted RNA as the starting material).

FMDV genome is organized in ~8.3kb single-stranded RNA. Our assay targets a highly conserved genomic region in the RdRp gene.

Target	FMDV (RdRp)	IC (Internal Control)
Detection Channel	FAM	HEX

The kit is supplied with an extraction Internal Control, which is added to the sample in the lysis step during RNA extraction. This approach ensures that the extraction and amplification steps are accounted for, thus eliminating the possibility of false negatives due to erroneous extraction and/or amplification.

## B. Kit Contents and Storage

The kit comes in a package of five tubes, as indicated in the following table. All components must be stored at -20°C. The MMS Oligo Mix should not be exposed to light for extended periods of time.

Component	Volume (uL)
One-Step RT-qPCR Master Mix	1,000
MMS Oligo Mix	200
Negative Control	100
Positive Control	100
Extraction Internal Control (IC)	1,000

### C. Protocol

1. Extract RNA or total nucleic acids from each sample as in your laboratory's approved internal protocol. However, during extraction, mix 10 $\mu$ L of the Extraction Internal control (IC) provided in the kit with each sample AFTER adding the lysis buffer. Nucleic acid should be used immediately after extraction (within 30 minutes) or stored for up to a week at -20°C if stored immediately after extraction.
2. Thaw all components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. **Do NOT exceed 3 freeze-thaw cycles!**
3. Prepare a reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):
  - a. 10  $\mu$ L of One-Step RT-qPCR Master Mix / reaction
  - b. 2  $\mu$ L of MMS Oligo Mix / reaction

#### Sample calculation

Component	Per 1 reaction	X10 reactions (+5% overage)
One-Step RT-qPCR Master Mix	10 $\mu$ L	105 $\mu$ L
MMS Oligo Mix	2 $\mu$ L	21 $\mu$ L

**NOTE: Don't forget to figure in the positive and negative samples in your calculations!**

4. Transfer 12  $\mu$ L of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.
5. Add 8  $\mu$ L of each sample's extracted nucleic acid to its designated well; add 8  $\mu$ L of the positive and negative controls to their designated wells.
6. Seal the plate with optical cap strips or an optical adhesive seal.
7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	FMDV	IC
Detection Channel	FAM	HEX

8. On the software, define your Positive and Negative Controls.
9. Run the Real-Time PCR thermal cycler for 45 cycles as follows:
  - Step1: 2 minutes - 25°C (the UNG step)
  - Step2: 10 minutes - 50°C (the RT step)
  - Step3: 2 minutes - 95°C (initial polymerase activation)
  - Step4: 10 seconds - 95°C (denaturation)
  - Step5: 30 seconds - 60°C (annealing/extension) → **DATA COLLECTION**
  - Step6: go to step4 (X39 times)
10. Analyze the results according to your thermal cycler's software. Use your judgement to set/modify the threshold of detection for each filter and/or omit amplification artifacts (e.g. non-sigmoidal amplification curve).

## D. Data Interpretation

### Sample calling

For the FMDV-specific target, the acceptable cycle threshold of detection is Ct <40, which means that Ct ≥40 should be considered as non-detected. If the FMDV-specific target was undetected, call the sample negative only if the Extraction Internal Control Ct is below 35. If it is greater than 35, there may have been an issue with the extraction, and we strongly recommend re-extracting the sample.

The following table summarizes our sample calling recommendations. A positive (+) sign indicates a Ct < 40 whereas a negative (–) sign indicates no amplification.

	<b>FMDV (FAM) CT</b>	<b>IC (HEX) CT</b>	<b>INTERPRETATION</b>
CASE 1	+	Any	<b>Positive infection</b>
CASE 2	–	≤35	<b>No infection</b>
CASE 3	–	>35	<b>Extraction failed; redo extraction</b>

### Run validity

For the run to be valid, the Negative Control must return a negative infection result, whereas the Positive Control must return a positive infection result (see sample calling above). If not, the whole run is considered invalid.

## **E. Support**

For questions, suggestions, or technical support, feel free to contact us by email on:

[support@havensci.com](mailto:support@havensci.com)

## **F. Quality Control**

Each lot is tested against predetermined parameters to ensure consistent performance among all lots.