MMS FMDV qPCR Detection Assay

Product Manual

Version 2.0

This product is intended for research use only.

Contents

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. Intended Use

This kit is intended for detection of all known variants of the FMD Virus with extracted RNA as the starting material. The kit does *NOT* include reagents for RNA extraction. See (Specimen Preparation) section for our recommended RNA extraction kits for each type of biological specimen.

It should be noted that this kit is intended for research use only. Please do not use it for human medical diagnostics.

C. 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)
RT-qPCR Master Mix	1,000
MMS Oligo Mix	200
Negative Control	100
Positive Control	100
Internal Control (IC)	1,000

D. Required Materials That Are Not Included in the Kit

- Real-time PCR thermal cycler (see next section: Compatible Instruments)
- PCR plates compatible with your real-time PCR thermal cycler
- Optical adhesive seal and an applicator to seal off the PCR plate
- PCR plate microcentrifuge
- Pipettes and filtered pipette tips in the range of 1–1,000µL
- 1.5mL or 2.0mL microcentrifuge tubes
- Microcentrifuge with 1.5mL/2.0mL rotor
- (highly recommended) Ice or a cooled PCR stand/rack
- (optional) RNase-free water

E. Compatible Instruments

Our kit is compatible with commercially available open system real-time PCR thermal cyclers that can detect fluorescence at wavelengths 520nm (e.g. FAM) and 535nm (e.g. HEX, VIC, etc.). The following systems are all compatible with our assay:

- 7500 Fast Real-Time PCR System (ABI, 4351106)
- QuantStudio™ 5 Real-Time PCR System (ABI, A28574)
- QuantStudio™ 7 Flex Real-Time PCR System (ABI, 4485688)
- CFX96™ Real-Time PCR Detection System (Bio-Rad, 185-5096)
- CFX Opus 96 Real-Time PCR System (Bio-Rad, 12011319)
- Rotor-Gene Q 5plex Platform (Qiagen, 9001570)
- LightCycler® 480 Instrument (Riche Diagnostics, 05015278001)

Please consult us about the compatibility of your thermal cycler by sending an email to support@havensci.com.

F. Good PCR Practices

Contamination is the worst enemy of PCR. Although the positive and negative controls in our kit can help in detecting contamination, dealing with contamination could prove troublesome and costly. Here, we list a few measures that could help you avoid contamination:

- Have physically separate workstation for PCR preparation, sample loading, and nucleic acid extraction. Each workstation should have its own dedicated pipettes and other small instruments, and should preferably be in a separate room.
- Always work under a cabinet (laminar flow for PCR preparation, and safety cabinet for nucleic acid extraction and sample loading).
- Use filtered tips. They are more expensive, but the small extra cost may save you valuable time, effort, and money down the line.
- If you need to analyze the PCR product after PCR cycling has finished, never unseal the plate near your PCR preparation or sample loading workstations. Even the smallest of aerosols could contain tens of millions of PCR templates.

G. Specimen Preparation

Make sure to have the extracted RNA or total nucleic acid ready before proceeding to PCR preparation in the next section. We recommend using the following extraction kits depending on your biological specimen:

Specimen Type	Extraction Kit
Animal Tissue (Silica columns)	RNAbler - Cells and Tissue, Cat. No. RE95050
Animal Tissue (Magnetic beads)	Mag RNAbler - Cells and Tissue, Cat. No. RE99032-PF
Animal Blood (Silica columns)	Viral TNAbler, Cat. No. TE98050
Animal Blood (Magnetic beads)	Mag Viral TNAbler (Prefilled), Cat. No. TE99032-PF (Optimized on our MagEX 32 automated extraction system, Cat. No. NE032)

H. Protocols

1. Extract RNA using our kits outlined in the previous section (Specimen Preparation), based on your sample type. However, during extraction, mix 10µL of the Extraction Internal control (IC) provided in the kit with each sample **AFTER** adding the lysis buffer. The extracted nucleic acid should be used immediately after extraction (within 30 minutes) or stored at -20°C 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 the reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):

    a. *10 µL of RT-qPCR Master Mix / reaction*
    b. *2 µL of the MMS Oligo Mix / reaction*

Example

Component	Per 1 reaction	X10 reactions (+5% overage)
RT-qPCR Master Mix	*10 µL*	*105 µL*
MMS Oligo Mix	*2 µL*	*21 µL*

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

4. Transfer 12 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

NOTE: If you wish to add a smaller sample volume, fill to 20µL using RNase-free water

6. Seal the plate with optical cap strips or an optical adhesive seal. Spin down the plate to collect all components to the bottom of the plate.

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.

1. 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 - 55°C (annealing/extension)* → ***DATA COLLECTION***

Step6: *go to step4 (X39 times)*

9. 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, etc.).

I. 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.

	FMDV (FAM) CT	IC (HEX) CT	INTERPRETATION
CASE1	+	Any	**Positive infection**
CASE 2	–	≤35	**No infection**
CASE 3	–	>35	**Extraction failed; redo extraction**

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.

J. Support

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

support@havensci.com

K. Quality Control

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