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SN FMDV ENSURE

- One Step Multiplex RT-PCR Kit -

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SN FMDV Ensure

one step multiplex RT-PCR kit

Description of the Product:

The SN FMDV Ensure is a ready-to-use kit designed for the detection of Foot and Mouth Disease Virus (FMDV) using Polymerase Chain Reaction (PCR) in a Real-Time PCR machine. It enables the rapid and reliable identification of the virus, determining its presence or absence with high sensitivity. The Specific Master mix contains reagents and enzymes for the specific amplification of FMDV genes **(3DP,5'UTR)and IC 18s rRNA Gene** to generate amplified product and for the direct detection of the specify amplicon in fluorescence channel **FAM(5'UTR Gene), Cy5(3DP Gene),Rox(18s rRNA)** of the Real Time PCR.

Intended to use:

RT-PCR based diagnostic kit for the detection of FMDV from Sewage, Milk, feed and clinical samples etc. from cattle's, swine, sheep, goats, and other cloven-hoofed animals. It is recommended to use standard viral RNA extraction kit (spin column based or magnetic bead based) for isolation of viral RNA. The kit will also work on the automated RNA purification system. The kit intended to use by trained laboratory technician.

1. Contents of Kit:

Components	Color Code	Volume
SN FMDV Ensure RT Master Mix (MM)	Natural	1000µL
PP Mix	Amber Tube	200µL
Positive control (PC)	Blue	100µL
Nuclease free water (NC)	White	100µL

***The Positive Control contains both targets; FMDV 3DP & 5'UTR Gene.**
All Vials have Color Coder tops to distinguish between different reagents.

Storage:

The kit is shipped and stored at -20^{0}C

❖ Sample which can be used for extraction:

Sewage ,Milk and feed samples, tissue etc samples

2. Procedure:

The performance of the SN FMDV Ensure depends on the amount and quality of template RNA purified from the clinical samples.

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The extraction protocol should be performed following manufacturer's instructions or an internally validated protocol.

3. SN FMDV Ensures PCR Master Mix preparation:

1. Prepare the master mix as per table mentioned below. Positive control (PC) and Negative test control (NC/NTC) should be included in every set up.

Color Code	Components	Volume (µL)/Reaction	Volume (µL) for 100 Reaction
Natural	SN FMDV Ensure RT Master Mix (MM)	10 µL	1000 µL
Amber Tube	PP Mix	2 µL	200 µL
Total Volume		**12 µL**	1200 µL

Add each component to multiple number of samples to be processed and prepare the master mix. It is advisable to prepare extra mix for one sample for any pipetting related error or dead volumes.

Note:

1. Dispense 12 µL from above master mix in separate tubes/wells
2. Add 8 µL of isolated RNA from patients sample separately to each tube

Once dispensed, seal the plate with optically clear sealers or close the tubes-strips, centrifuge the tubes/strips or plates briefly and place them in the instrument.

4. Thermal Profile: (Setting of RT-PCR)

Referring to the instrument manual, set on the dedicated software for the parameters of thermal cycle.

Steps	Temp	Time	Cycle
Hold 1	50 ^{0}C	15 min	1
Hold 2	95 ^{0}C	2 min	1
Cycling	95 ^{0}C	15 secs	49
	60 ^{0}C	30 secs	

5. Fluorescence Setup:

Target	Applied Biosystem 7500	BioRad CFX-96	Rotor Gene Q
3DP gene	Cy5	Cy5	Purple Channel
5'UTR Gene	FAM	FAM	Blue Channel
IC	VIC	ROX	Orange Channel

Note:

The Kit is compatible with; Rotor Gene™ 3000/6000, ABI Step One, 7500/FAST, Quant studio, BioRad CFX 96, Roche 480 and other Real Time PCR machine available.

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6. Precautions for PCR:

The following aspects should always be taken care of:

a) Store positive material (Specimens, Positive controls & amplicons) separately from all other reagents and add it to the reaction mix in a separate area.
b) Thaw all components thoroughly at room temperature before starting the assay.
c) When thawed, mix the components and centrifuge briefly.
d) Work quickly on ice or in the Cooling Block.
e) All the reagents including the NTC (except for PCs & specimens) should be mixed & dispensed in pre-mix area.
f) All the controls & specimens should be mixed & dispensed in extraction area.
g) Use pipette tips with filters only.
h) Always use disposable powder-free gloves.
i) Discard samples and assay waste according to your local safety regulations.
j) Do not open the reaction tubes or plates post amplification.

7. Additionally, required Materials and Devices

I. Appropriate 3 or more channels RT-PCR instrument, compatible with the fluorophores used in this test.
II. Appropriate nucleic acid extraction system or kit. It is recommended to use commercialized extraction kit extracting RNA.
III. Vortex mixer.
IV. Centrifuge with a rotor for 2 mL micro centrifuge tubes.
V. Pipette tips with filters (disposable).
VI. Powder-free gloves (disposable).
VII. Class II Biological Safety Cabinet (BSC), ideally in a BSL-2 facility.

8. Result Interpretation:

Amplification of PC suggest absence of any inhibition during amplification. Amplification of IC in samples suggest proper RNA extraction. Amplification in Cy5 and FAM channels indicate presence of FMDV in sample. Due to competition and limiting factors, samples with high viral copy may not show amplification in ROX(IC) channel and the curves may not be a proper sigmoid. Results can be analyzed with the help as mentioned below:

9. Data analysis:

Ct cut off value for Cy5 and FAM is ≤34, Sigmoidal curves.

Note:
Ct value in any sample/well >34 should be considered as below detection limit of the kit

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10. Result Interpretation table:

SL.NO	ROX (internal control) IC	FAM 5'UTR Gene	Cy5 3DP	Result Interpretation
1.	+/-	+	+	FMDV RNA detected
2.	+/-	+	-	FMDV RNA detected*, require further investigation
3.	+/-	-	+	FMDV RNA detected*, require further investigation
4.	+	-	-	All targets are valid, FMDV RNA NOT detected
5	-	-	-	Invalid repeat sampling/extraction/amplification

12. Trouble shooting:

* Retest the sample if the retest results are same, then it could be presumed as positive.

11. Analytical Performance:

Evaluation of the kit on the confirm positives and negative specimen showed 100% specificity and sensitivity. The kit can detect 10 copies per µl of FMDV RNA from clinical sample.

Problem	Possible Cause	Recommendation
No signal in all samples including positive control	Error in Master mix preparation Inhibitors added Probe degradation Positive control degradation Omitted components Instrument setting error	Check the dispensing volume during preparation of master mixture Repeat the extraction step with new sample, use a new probe reagent, use a new positive control Verify each component, repeat the RT-PCR mixture preparation Check the position setting for the positive control on the instruments. Check the Thermal cycle settings on sample instrument
Diverse intensity of fluorescent signals	Pipetting error Contamination in the outer surface of PCR tubes or Plate	Make sure that the equal volume of reactants is added in each tube or plate. Wear gloves during the experiment
Weak or no fluorescent signal in samples only	Poor RNA quality, Insufficient volume of RNA	Use recommended kit for RNA extraction and store extracted RNA at -80°C, Repeat PCR reaction with correct volume of RNA Fluorescence
No template Control (NTC) showing Amplification	Amplification of FMDV genes in a no Template Control indicates contamination in one or more of the reagents, incorrect placement of a plate or tubes into the RT-PCR instrument, or pipetting error	Repeat the test by using 2 NTC. If NTC fails again, then investigation should be conducted to identify possible causes of error