Foot-and-Mouth Disease Virus (FMDV)

RT-PCR Kit (96 reactions)

For In Vitro Diagnostic Use only

User Manual

Manufactured By:

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1- Foot - and - mouth disease:

Foot - and - mouth disease (FMD) is a very contagious disease for cow, goat, pig and sheep which cause decrease in production and economic loss. The disease agent is a member of Picronaviridae family and a prototype of Aphthovirus genus. Seven serotypes of this virus including A, O, C, Asia1, SAT1, SAT2 and SAT3 have been reported. There is a need for detection of FMD free animals to distinguish them from carriers.

All serotypes of this virus are detected with this kit using one step RT-PCR in short time. In this kit RT (cDNA amplification) and PCR are carried out in one step in one tube and there is no need for cDNA construction separately.

2- Storage:

All kit's components must be kept at -20°C. In this condition kit's materials are stable and usable until expire date.

3- Kit contents:

1- Pink micro-centrifuge tube containing blue lyophilized material.
2- Blue capped micro-centrifuge tube containing RT-PCR buffer solvent
3- Red capped micro-centrifuge tube containing positive control solution
4- Yellow capped micro-centrifuge tube containing DEPC-ddH_2O

4- Required equipments and materials:

1- Standard tabletop micro-centrifuge (13000 rpm)
2- Vortexer
3- Thermocycler
4- Electrophorese equipment and UV light box
5- RNA extraction kit
6- 0.5-10 and 10-10 µl sampler
7- Sterile tips and gloves

5- Protocol:

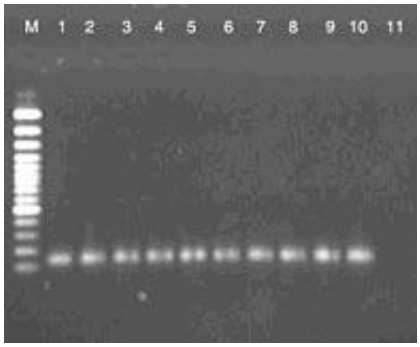
1- Prepare tissue samples in standard condition and transport to the lab on ice and storage at -20 before usage.
2- Extract virus RNA from samples using standard kits. (RNA extraction kit from Roche and QIAGEN are recommended)
3- Take out the pink tubes from -20°C and mark as positive control, negative control and sample.
4- Add 18 µl RT-PCR buffer solvent to each tube.
5- Add 2 µl of extracted RNA to the sample tube.
6- Add 2 µl of DEPC-ddH_2O to the negative control tube.
7- Add 2 µl of positive control solution to the positive control tube.
8- Vortex the contents of the tubes (make sure that the lyophilized material to be dissolved completely), spin the tube and put into the thermocycler.
9- PCR program is as follow.

RT-PCR reaction		Temperature (ºC)	Time
1 cycle	Reverse Transcription (RT)	45	30 min
	Inactivation of reverse transcriptase	94	5 min
30 cycles	Denaturation	94	30 sec
	Annealing	55	30 sec
	Extension	72	30 sec
1 cycle	Final extension	72	10 min

10- Load 10 µl of the PCR reaction on a 1% agarose gel along with a suitable DNA size marker and electrophorese (90 volt, 40-45 min). Stain the gel with ethidium bromide and observe the result on a UV light box.

6- Result description:

If the RNA extraction, RT-PCR and electrophoresis have been carried out properly, there should be a DNA band of about 130 bp in the positive control and no band in the negative control (Fig). The results for the samples should be compared and described according to the results for negative and positive controls.



M: DNA size marker; 1-9: PCR product of the samples (131 bp)
10: positive control; 11: negative control

7- Precautions:

- Wear gloves during all stages
- Do not use expired materials
- Do not use materials from other detection kits in this kit
- Follow the protocol steps carefully
- In the case of multiple sample detection, be careful to avoid cross contamination

8- Troubleshooting:

- If there is nonspecific bands in the gel, dilute the template RNA with DEPC-ddH_2O (1/5)
- Preparation of the RT-PCR reaction in the room temperature may cause nonspecific PCR product(s). Therefore, preparation of reaction mixture on the ice is recommended.

For Further Questions and Problems, please Contact our Technical Support at najmbiotech.ltd@gmail.com