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Genotype B5 of Enterovirus 71(EV71-B5)Real Time RT-PCR Kit User Manual

LT024030RQ50

For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/ Chromo4; LightCycler®480 instrument

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50 −20 °C CE

1. Intended Use

By using real time PCR systems, Genotype B5 of Enterovirus 71(EV71-B5)Real Time RT-PCR Kit is used for the detection of Enterovirus 71 genotype B5 in samples like nasal and pharyngeal secretions, sputum, provoked sputum, stool, C.S.F , serum and etc.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Enterovirus 71 (EV71), one of the major causative agents for hand, foot and mouth disease (HFMD), is sometimes associated with severe central nervous system diseases. In 1997, in Malaysia and Japan, and in 1998 in Taiwan, there were HFMD epidemics involving sudden deaths among young children, and EV71 was isolated from the HFMD patients, including the fatal cases. The nucleotide sequences of each EV71 isolate were determined and compared by phylogenetical analysis. EV71 strains from previously reported epidemics belonged to genotype A-1, while those from recent epidemics could be divided into two genotypes, A-2 and B. The genotype B is subdivided into B1-B5.

The kit contains a specific ready-to-use system for the detection of the genotype B of EV71 using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a Super Mix for the specific amplification of the virus RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems´ optical unit during the PCR. The detection of amplified virus DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. An external positive control is supplied.

4. Kit Contents

Ref.	Type of reagent	Presentation	50rxns
1	EV71-B5 Super Mix	1 vial, 480µl	x2
2	RT-PCR Enzyme Mix	1 vial, 28µl	x2
3	Molecular Grade Water	1 vial, 400µl	x2
4	EV71-B5 Positive Control	1 vial, 30µl	x2

Analysis sensitivity: 1×10^4copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided.
- Cool all reagents during the working steps.
- Super Mix and Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. ⚠Warnings and Precaution

Carefully read this instruction before starting the procedure.

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

8. Sample Collection, Storage and transport

- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.

9. Procedure

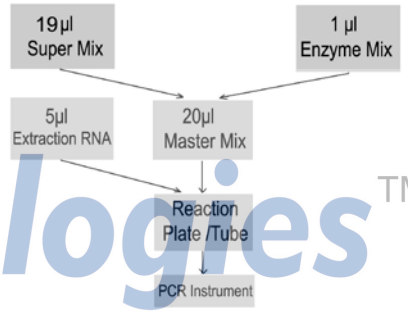
9.1 RNA-Extraction

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
RNA Isolation Kit	EM-0100/EM-2100	Life Tech
QIAamp Viral RNA Mini extraction Kit (50)	52904	QIAGEN

9.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **20µl** Master Mix with micropipets of sterile filter tips to each of the real time PCR reaction plate/tubes. Separately add **5µl** RNA sample template, positive and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

45°C for 10min	1cycle
95°C for 15min	1cycle
95°C for 15sec, 60°C for 1min (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
FAM	Target Nucleic Acid

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher**.

10. Threshold setting: just above the maximum level of molecular grade water.

11. Quality control:

Negative control,and positive control must be performed correctly, otherwise the sample results is invalid.

Control \ Channel	Ct value
	FAM
Molecular Grade Water	UNDET
Positive Control(qualitative assay)	≤35

12. Data Analysis and Interpretation

The following sample results are possible:

	Ct value	Result Analysis
	FAM	
1#	UNDET	Below the detection limit or negative
2#	≤38	Positive;
3#	38~40	Re-test; if it is still 38~40, report as 1#