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HCV Genotype Real Time RT-PCR Kit User Manual

LT029000RH

For use with ABIPrism®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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Σ 25 −20 °C CE

1. Intended Use

HCV genotype real time RT-PCR kit is used for the detection of HCV genotype 1 in blood serum by using real time PCR systems.

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 (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Hepatitis C virus has at least six forms or genotypes. HCV genotypes and subtypes are distributed variously in different parts of the world. Genotypes 1-3 are widely distributed throughout the world. Subtype 1a is prevalent in North and South America, Europe, and Australia. Subtype 1b is common in North America and Europe, and is also found in parts of Asia. Genotype 2 exists in most developed countries, and is less common than genotype 1. Some studies suggest that different types of HCV may be related to different transmission routes. HCV genotype 1 is significantly associated with human immunodeficiency virus. Genotype 1 is related to a poor response to treatment. Genotyping can help doctor determine an appropriate hepatitis C treatment and how long treatment should be given.

HCV genotype real time RT-PCR kit contains a specific ready-to-use system for the detection of HCV genotype 1 by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The master contains Super Mix for the specific amplification of HCV RNA and HCV genotype1 RNA. Super Mix A is specific for HCV RNA; Super Mix B is specific for HCV genotype1 RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT): HCV RNA is transcribed into cDNA. Then, a thermostable DNA polymerase is used to amplify the specific gene fragments by polymerase chain reaction (PCR).Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified HCV DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1.

4. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	HCV Super Mix A	1 vial, 480µl
2	HCV Super Mix B	1 vial, 480µl
3	RT-PCR Enzyme Mix	1 vial, 54µl
4	Molecular Grade Water	1 vial, 400µl
5	HCV Positive control A	1 vial, 60µl
6	HCV Positive control B	1 vial, 60µl

Analysis sensitivity: 5×10^3IU/ml; LOQ: 1×10^4～1×10^8copies/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, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super 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, and 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.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9. Procedure

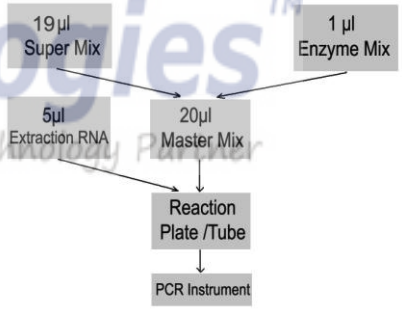
9.1 RNA-Extraction

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

RNA Extraction Kit GEN 52-904 LT

9.2 RT-PCR Protocol

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



1）The volumes of Super 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 Super Mix** with micropipets of sterile filter tips to several Real time PCR reaction tubes. Separately add **5µl** RNA sample，positive control A, positive control B and negative control to different reaction tubes. Immediately close the 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,58°C for 1min (Fluorescence measured at 58°C)	40cycles

Selection of fluorescence channels	
Reaction Mix A：FAM	HCV
Reaction Mix B：FAM	HCV genotype I

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.

Super Mix Control	Ct value	
	Super Mix A	Super Mix B
Molecular Grade Water	UNDET	UNDET
HCV positive control A	≤35	UNDET
HCV positive control B	≤35	≤35

12. Data Analysis and Interpretation

Negative or positive judgement：

	Ct Value	Negative or Positive
1	UNDET	Negative "—"
2	≤38	Positive "+"
3	38～40	Re-test; If it is still 38~40, then Negative "—"

The following results are possible:

Results	Super Mix A	Super Mix B	| Ct Value of Super Mix A—Ct Value of Super Mix B |	Conclusion
1	—	—	—	HCV Negative
2	+	+	≤3.5	HCV Positive and Genotype I
3	+	+	>3.5	HCV Positive but not Genotype I
4	+	—	—	HCV Positive but not Genotype I