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PCR / RT-PCR Kit User's Manual

(48T)

Cat# BSB02M1D

For HCV PCR Fluorescence Quantitative Detection
(With Internal control and Magabeads Extraction)

Preface

Hepatitis C Virus is considered to be the principal etiologic agent responsible for 90-95% of the cases of post-transfusion non-A and non-B hepatitis.1,2 HCV is a single-stranded, positive sense RNA virus with a genome of approximately 10,000 nucleotides coding for 3,000 amino acids.[1] As a blood-borne virus, HCV can be transmitted by blood and blood products. The global prevalence of HCV infection, as determined by immunoserology, ranges from 0.6% in Canada to 1.5% in Japan.[2]

Serological screening assays have greatly reduced, but not completely eliminated, the risk of transmitting viral infections by transfusion of blood products[3-6]. Recent studies indicate that nucleic acid-based amplification tests for HCV RNA will allow detection of HCV infection earlier than the current antibody based tests. Nucleic acid testing of whole blood donations has been in place in the United States since 1999 under Investigational New Drug Application. Nucleic acid-based tests can detect the units of virus donated by carriers who do not seroconvert or who lack antibodies to serological markers normally detected by immunological assays.

This fluorescence Detection Kit is adapted for many kinds of Real Time PCR Detection Instrument., specifically adapted for Line-Gene I&II Real-time PCR Detection System.

HCV RNA is reverse transcribed and a specific fragment is amplified with specific primers in a one-step RT-PCR reaction. The products are detected by using a specific Taqman-MGB Probes. This Taqman-MGB Probes is labeled at the 5'-end with FAM report dye, and the 3'-end by NFQ(Non Fluorescent Quencher)-MGB.

An Internal Control is supplied. This allows the user both to control the RNA isolation procedure and to check for possible PCR inhibition. For this application, add 1 μl the Internal Control to the isolation per sample.

Ingredients

Ingredients			Volume	Quantity
1	Virus RNA Extraction Reagent	Lysis Buffer	4.8ml	1 Bottle(s)
2		Wash Buffer Ⅰ	15.6ml	1 Bottle (s)
3		Wash Buffer Ⅱ	5.2ml	1 Bottle (s)
4		Wash Buffer Ⅲ	24ml	1 Bottle (s)
5		Elution Buffer	2.4ml	1 Bottle (s)
6		Magnetic Beads	480μl	1 Tube(s)
7		Proteinase K	480μl	1 Tube(s)
8		Acryl Carrier	96μl	1 Tube(s)
9	PCR Reagent	RT-PCR MIX	720μl	1 Tube(s)
10		Mn2+	96μl	1 Tube(s)
11		HCV Probe Mix 1	96μl	1 Tube(s)
12		Internal control	48μl	1 Tube(s)
13		Negative control	200μl	1 Tube(s)
14		Positive control	200μl	1 Tube(s)
15		Standard control 1: 5.0×10^{7}copies/ml	100μl	1 Tube(s)
16		Standard control 2: 5.0×10^{6}copies/ml	100μl	1 Tube(s)
17		Standard control 3: 5.0×10^{5}copies/ml	100μl	1 Tube(s)
18		Standard control 4: 5.0×10^{4}copies/ml	100μl	1 Tube(s)

Applied instrument

Line-Gene Series Real-time PCR detection system.

Storage and period of validity

Except for Proteinase K and Acryl Carrier store at -20℃, Virus RNA Extraction Reagent store at room temperature, PCR Reagent store at -20℃. The kit can be stored for up to 12 months if all components are kept in the manner above.

Additional required reagents and equipment

1. Ethanol, Nuclease-free aerosol-preventive pipette tips.
2. Sterile centrifuge (Eppendorf) tube for preparing, 0.2 ml real-time PCR tube.
3. Vortex shaker, dry bath, centrifuge and magnetic rack etc.

Specimen Collection and Storage

1. EDTA, CPD, CPDA-1, CP2D, ACD-A and 4% Sodium Citrate may be used with this kit.
2. Blood collected in EDTA may be stored at 2～30℃ for up to 72 hours from time of draw, followed by an additional two days at 2～8℃. For storage longer than five days, remove the plasma from the red blood cells by centrifugation at 800～1600xg for 20 minutes. Following removal, plasma may be stored at 2～8℃ for an additional seven days. Alternatively, plasma may be stored at -18℃ for up to one month.
3. Blood collected in CPD, CPDA-1, or CP2D may be stored for up to 72 hours at1～24℃. Following centrifugation of the CPD, CPDA-1, or CP2D samples at 800-1600xg for 20 minutes, plasma may be stored at 1～6℃ for an additional 7 days from the date the plasma was removed from the red blood cells. Plasma separated from the cells may be stored at-18℃ for up to one month.
4. ACD-A or 4% sodium citrate anticoagulated apheresis plasma can be stored at 1～6℃ for up to 6 hours, followed by subsequent storage at-18℃ for up to one month.
5. Do not freeze whole blood.
6. Heparin has been shown to inhibit PCR. Use of heparinized specimens is not recommended.
7. Warm pooled or individual donor specimens to room temperature before using.
8. Covered Archive Plates may be stored at 2 – 8℃ for up to 7 days from the date the plasma was removed from the red blood cells.
9. No adverse effect on assay performance was observed when plasma specimens were subjected to three freeze-thaw cycles.
10. Thaw frozen specimens at room temperature before using.
11. The user should validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.
12. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

Protocol

1. Sample extraction

Add 10.4 ml ethanol to Wash Buffer Ⅰ, add 20.8ml ethanol to Wash Buffer Ⅱ, Mix gently for 2 mins.

1.1 Add 10 μl Proteinase K, 2μl Acryl Carrier, 100μl Lysis Buffer（If precipitates have formed Place the bottle in a warm water bath（< 60℃) and shake the bottle approximately every 10 minutes until the precipitates are dissolved）and 100μl of serum sample (include Specimen, Positive Control, and Negative Control) into the 1.5ml centrifuge tube，mix thoroughly and incubate at 55℃ for 10mins.

1.2 Add 120 μl of ethanol and 10 μl of magnetic beads(mix well before use), Mix gently for 10mins at room temperature, centrifuge the tubes for a short while.

1.3 Put it on magnetic rack for 1 minute. Then discard the supernatant.

1.4 Add 500 μl of Wash buffer Ⅰ and vortex for 15 seconds. Centrifuge the tube for a short while. Put it on magnetic rack for 1 minute. Then discard the clarified supernatant.

1.5 Add 500 μl of Wash buffer Ⅱ and vortex for 15 seconds. Centrifuge the tube for a short while. Put it on magnetic rack for 1 minute. Then discard the clarified supernatant. Meanwhile, Open the cap and keep the 1.5ml centrifuge tube still on the magnetic rack.

1.6 Add 500 μl of Wash buffer Ⅲ, Do not rush the magnetic beads down ; Then discard the clarified supernatant at once.

1.7 Add 50μl of Elution buffer and vortex for 30 seconds.

1.8 Incubate at 55℃ for 5 minutes. Waving the tube light ly twice during this time in order to dissolve the RNA. Centrifuge the tube for a short while. And then put it on magnetic rack for 2 minutes, and keep the supernatant to an RNase-free tube for use later.

2. PCR reaction mixtures prepare:

Define the experimental protocol before preparing the solutions. Calculate the number of reactions needed plus one additional reaction for the HCV RT-PCR MIX. Proceed as described below for a 40 μl standard reaction when preparing the reaction mixtures.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>

1. Reagent prepare:

Note: Thaw out the reagents at room temperature. Before preparing RT-PCR reagents, mix gently and centrifuge all reagents for a few seconds.

Make RT-PCR reagents according to the quantity of sample and controls as below (n tests add an extra blank control).

The volumes per tube are mentioned below:

<table>
<tr><th>Component</th><th>RT-PCR MIX</th><th>Mn2+</th><th>HCV Probe Mix 1</th><th>Internal control</th></tr>
<tr><td>Dosage/ test</td><td>15.0 μl</td><td>2.0μl</td><td>2.0μl</td><td>1.0μl</td></tr>
<tr><td>Dosage</td><td>（n+1）× 15 μl</td><td>（n+1）× 2.0 μl</td><td>（n+1）× 2.0 μl</td><td>（n+1） μl</td></tr>
</table>

</td></tr>
<tr><td>2</td><td>

- Mix gently, centrifuge for a short time and pipette 20μl the mixture into the corresponding Real-time PCR tube.
- Add 20 μl of the corresponding HCV RNA template (Specimen; controls).
- Add 20 μl of the standard control 1 to 4.

</td></tr>
<tr><td>3</td><td>

- Cap each tube, mix gently and centrifuge for a short time.
- Centrifuge at 700 × g for 5 s (3000 rpm in a standard benchtop microcentrifuge).

***Note*:** Place the centrifuge adapters in a balanced arrangement within the centrifuge.

</td></tr>
<tr><td>4</td><td>Place the PCR tube in the Real Time PCR Detection Instrument.</td></tr>
<tr><td>5</td><td>

Set reaction procedure as following:

90℃：30 sec；
61℃：20 min；
95℃： 1 min；
95℃：15 sec；
60℃：1 min；
} 45cycles

Select the fluorescent channel of instrument for testing:

Choose F1（FAM）and F2 (HEX) channels to collect fluorescent signals.

Before running Line-Gene Series Real-time PCR detection system, set fluorescent signals detecting at 60℃ for 10 seconds, and then adjust gain to make the F1（FAM）and F2 (HEX) background between 20-30.

</td></tr>
</table>

Result analysis and judgments

Perform data analysis, as described in the Real Time PCR Detection Instrument Operator's Manual. Analysis method Use of the Fit points method. This renders the method independent from user-born influences.

Quality Control

1) The Ct value of the Negative control must be ∞.
2) The Correlation of standard curve must be ≤-0.97.
3) The Ct value of the Internal control must be ＜36.
4) The Ct value of the positive control must be ＜35.

Note

a) This kit is for research use only.
b) Before you begin, you should read this user's manual carefully.
Lysis buffer and wash buffer Ⅰ will crystallize if the room temperature is low.You must place the bottle in a warm water bath at 37℃ and shake the bottle approximately eyery 5mins until the crystallization disappear .
c) Prepare appropriate aliquots of the kit solutions and keep them separate from other reagents in the laboratory.
d) The use of nuclease-free lab ware (e.g. pipettes, pipette tips, reactions vials)as well as.
e) Wearing gloves when performing the assay.
f) To avoid cross-contamination of samples and reagents use fresh aerosol-preventive pipette tips for all pipetting steps.
g) To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube instead of directly pipetting from stock solutions.
h) Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.
i) To minimize risk of carry-over contamination, it is worthwhile to physically separate the workplaces for RNA preparation.

References

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