Hepatitis C Virus Nucleic Acid Quantitative Detection Kit Manual

(Fluorescence quantitative PCR method)

【Product Name】

Generic name: Hepatitis C virus Nucleic Acid Quantitative Detection Kit (fluorescence quantitative PCR method)

【Packaging Specification】

24 tests/box

【Intended Use】

Hepatitis C is an infectious disease caused by hepatitis C virus (HCV), which is mainly transmitted through blood and damages liver. Chronic HCV infection can lead to chronic liver inflammation, necrosis and fibrosis. Some patients can develop into liver cirrhosis or hepatocellular carcinoma, which is extremely harmful to the health and life of patients. It has become a serious social and public health issue.

This kit is used for quantitative detection of hepatitis C virus (HCV) RNA in human serum or plasma samples covering HCV genotypes 1, 2, 3, 4, 5 and 6. The quantitative detection of HCV RNA in samples can be auxiliary for the diagnosis of hepatitis C and the therapeutic effect evaluation of antiviral therapy. The test result of this kit is not the only criterion for evaluating the patient's condition. Comprehensive analysis must be carried out in combination with clinical manifestations and other laboratory tests. This kit should not be used as a screening reagent for HCV blood screening.

【Detection Principle】

Specific primers and fluorescent probes were designed for the conserved hepatitis C virus (HCV) nucleic acid regions, and real-time fluorescent PCR technology was adopted to rapidly and quantitatively detect HCV RNA in samples. The kit provided an internal control, which is processed synchronously after being mixed with the sample to monitor the whole process of the experiment and effectively avoid the occurrence of false negative results.

【Kit Contents】

No.	Contents	Specification	Quantity	Ingredients
1	HCV-PCR reaction solution	672μL/vial	1	Primers, probes, dNTP, buffer system
2	RT-PCR polymerase	48μL/vial	1	Taq polymerase, reverse transcriptase
3	Internal Control	48μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate internal control gene fragments
4	HCV quantitative reference standard（1.0E+04IU/mL）	200μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate HCV gene fragments
5	HCV quantitative reference standard（1.0E+05IU/mL）	200μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate HCV gene fragments
6	HCV quantitative reference standard（1.0E+06IU/mL）	200μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate HCV gene fragments
7	HCV quantitative reference standard（1.0E+07IU/mL）	200μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate HCV gene fragments
8	HCV weak positive control	200μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate HCV gene fragments
9	HCV strong positive control	200μL/vial	1	Noninfectious in vitro transcribed RNA containing appropriate HCV gene fragments
10	HCV negative control	200μL/vial	1	Inactivated HCV positive serum

The components of different batch numbers in the kit cannot be used interchangeably.

【Storage conditions and validity period】

Stored at -20±5℃, the kit is valid for 8 months. It can be kept at 2-8℃ for 12 hours after opening. Avoid repeated freezing and thawing (Less than 4 times). Ice packs should be used during long distance transportation (less than 4 days). Production date and validity period are on the product label.

【Compatible Instruments】

ABI7500, STRATAGENE Mx3000p, TIB8600 etc.

【Sample Requirements】

1. Sample type: serum or plasma.
2. Sample collection

2.1 Serum:

Use a disposable sterile syringe to draw 2mL venous blood from the patient and inject

it into a sterile dry glass tube and place at room temperature (22 ~ 25 ℃). After 30 - 60 minutes, the blood sample can coagulate completely and precipitate serum spontaneously. Or directly use a horizontal centrifuge and centrifuge at 1,500rpm for 5 minutes. Pipette the upper layer serum and transfer to 1.5mL sterilized centrifuge tube.

2.2 Plasma

Use a disposable sterile syringe to draw 2mL venous blood from the patient and inject it into an anticoagulant glass tube containing EDTA (ethylene diamine tetraacetic acid) or sodium citrate. Turn it upside down for 5-10 times to mix the anticoagulant and venous blood. The blood sample can precipitate serum spontaneously after 5-10 minutes and then transfer to 1.5mL sterilized centrifuge tube.

3. Sample storage and transport

Sample can be used for immediate detection. Serum can be stored at – 15℃ no more than 3 months. Keep it below -70 ° C for long time preservation. Avoid repeated freezing and thawing. Samples should be transported in foam boxes and 0° C ice packs sealed.

【Procedure】

1. Reagent Preparation (Reagent Preparation Area)

1.1 After determining the number n of reaction tubes to be carried out (n = sample number + 7), take out the HCV-PCR reaction solution, and add n x 28μL HCV-PCR reaction solution and n x 2μL RT-PCR polymerase system into a centrifuge tube and vortex. Aliquot 30μL into each PCR reaction tube after spinning down briefly.

2. Nucleic acid Extraction (Sample Processing Area)

Pipet 200μL sample, negative control, HCV strong positive control, HCV weak positive control and 4 HCV quantitative reference standards then proceed with nucleic acid extraction. The commercial nucleic acid extraction kit is used to extract viral RNA from hepatitis C virus samples. 2μL internal control should be added to each sample (including quality controls and quantitative reference standards) during the extraction process, and the operation should be carried out according to the instruction of the kit.

3. PCR amplification

Add 20μL of sample, quality controls, quantitative reference standards and RNA extracted products to the prepared HCV reaction solution respectively. Flick the tube gently to mix well and avoid bubbles. Put into the fluorescence PCR instrument after spinning down briefly. Edit the sample information and amplification is carried out according to the following temperature profile.

Cycle Parameters: Stage 1: 42℃ ---- 30 min Stage 2: 95℃ ---- 3 min Stage 3 (10 cycles): 94℃ ----20 sec 55℃ ---- 20 sec 72℃ ---- 30 sec Stage 4 (35 cycles): 94℃ ----15 sec 60℃ ---- 20 sec	HCV detection fluorescein: FAM Internal control fluorescein: HEX Reaction Volume : 50μL Fluorescent signal collection: Stage 4: 60℃ ---- 20 sec Note: Stage 4: 60℃ ---- 20 sec, due to software issue of ABI7500, 20 sec setting is not available, 31 sec setting is acceptable.

4. Result analysis

4.1 Baseline setting: it can be set by the instrument automatically or users can adjust themselves according to the actual situation. Start value can be set at 1-3, End value can be set at 7-20.

4.2 Threshold setting: it can be set by the instrument automatically or manually set the threshold line just exceeds the peak of the normal negative control amplification curve.

4.3 Standard curve: input the concentration values of each quantitative reference standard into the corresponding detection hole position of the instrument, and the software automatically generates the standard curves.

5. Quality control

5.1 Negative control, HCV (FAM) Ct value ≥ 35 or "No Ct" and HEX Ct value ＜ 30 with nice log amplification curve.

5.2 HCV strong and weak positive control, FAM with nice log amplification curve. The strong positive control quantitative value is between 1.0E+06 and 1.0E+07（IU/mL）. The weak positive control quantitative value is between 1.0E+02 and 1.0E+03（IU/mL）. HEX Ct value ＜30.

5.3 HCV quantitative reference standards. FAM with nice log amplification curve. The linear correlation coefficient should be｜r｜≥0.98

The above requirements should be met in a single experiment, otherwise the results are invalid and retest is needed.

【Reference Value】

The detection lower limit for this kit is 25IU/mL, Ct reference value of internal control is <30.

【Results Interpretation】

1. If the sample detection value is between 5.0E+01 and 1.0E+08 IU/mL with nice log amplification curve, the detection value can be reported directly.

2. If the sample detection value is above 1.0E+08 IU/mL with nice log amplification curve, the detection result is reported as ＞1.0E+08 IU/mL. If accurate quantitative

result is needed, dilute the extracted sample below 1.0E+08 IU/mL and retest.
3. If the sample detection value is between 25IU/mL and 50IU/mL with nice log amplification curve, meanwhile, the internal control is positive and Ct value ＜30, it indicates that the virus load is low and detection results are for reference only.
4. If the sample detection value is lower than 25 IU/mL, meanwhile the internal control is positive and Ct value ＜30, it indicates that the HCV DNA concentration is lower than the detection lower limit of this kit.
5. If the sample detection value is lower than 25 IU/mL, meanwhile the internal control is abnormal (Ct value ＞ 30 or no Ct), it indicates the test is invalid and retest is needed.

【Detection Limitation】
1. The test results are for clinical reference only. The clinical diagnosis and treatment of patients should be comprehensively considered in combination with their symptoms, medical history, other laboratory examinations and treatment reactions.
2. Unreasonable process of sample collection, transfer, storage may lead to wrong detection results.
3. This kit is limited to the detection of human serum and plasma samples and is only applicable to the specified instruments.
4. The target sequence detected by the kit is hepatitis C virus gene, and the target sequence is highly conservative and stable. However, if gene mutation occurs at the target sequence, false negative results may occur.
5. The quantitative results of HCV RNA depend on the number of virus particles present in the sample, which may be affected by factors such as sample collection, processing, transportation, storage, patient's age, medical history and different stages of infection.
6. The results are for reference of clinical diagnosis only, and not the only criterion to confirm HCV infection. It should not be used for blood screening. Negative result only indicates that sample is below the minimum detectable concentration of this kit and should not be used to exclude the possibility of HCV infection.

【Kit Performance】
1. The coincidence rate of the negative and positive control was 100 %.
2. The limit of detection is 25IU/mL and the limit of quantitation is 50IU/mL. Limit of detection reference gradient dilution coefficient r ≥0.98
3. Linear range from 5.0E+01 to 1.0E+08IU/mL.
4. Accuracy references (strong positive reference and weak positive reference) were detected 20 times respectively. Positive detection rate was 100%. Meanwhile, the CV value within same batch and between different batches are both lower than 5%.
5. This kit covers HCV genotypes 1, 2, 3, 4, 5 and 6.
6. The kit has no cross reaction with hepatitis A virus, hepatitis B virus, human immunodeficiency virus, treponema pallidum, human cytomegalovirus, Epstein-Barr virus, herpes simplex virus, staphylococcus aureus and candida albicans pathogens.
7. Interfering factors such as free hemoglobin（≤200mg/ dL）, direct bilirubin （≤

30mg/ dL）, triglyceride （≤3g/ dL）had no effect on the detection.
8. Common antiviral drugs such as lamivudine, adefovir dipivoxil, ribavirin, peginterferon α-2a, peginterferon α-2b had no effect on the detection.

【Warnings and Precaution】

1. This product is for in vitro diagnosis purpose only.
2. Read this manual in detail before the assay, and the assay should be carried out by skilled personnel.
3. Use latex gloves or thin film gloves when handling the PCR tubes.
4. Avoid unnecessary repeated freezing and thawing and keep the PCR solution away from lights.
5. Thoroughly thaw the reagents and spin them down briefly before using.
6. It is recommended to use disposable centrifuge tubes and pipette tips without DNA enzyme and RNA enzyme.
7. Processing and handling of clinical specimens should be carried out in a biosafety cabinet.
8. After being spin down, the PCR tubes should avoid vortex when being loaded on the PCR instrument.
9. After amplification, the reaction tubes should be unloaded and sealed in a special plastic bag and disposed as medical wastes.
10. Do not mix-use the reagents from different batches. Use this kit before its expiration.
11. All components of this kit may be toxic. Keep away from mouth.

【Manufacturer】

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