Instructions for Use of Diagnostic Kit for Quantification of Hepatitis C Virus RNA (PCR-Fluorescence Probing)

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CE 2934

Diagnostic Kit for Quantification of Hepatitis C Virus RNA (PCR -Fluorescence Probing)	*DA0182*	*Large package, 48 tests/kit*

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1. Product Name

Diagnostic Kit for Quantification of Hepatitis C Virus RNA (PCR-Fluorescence Probing)

2. Package Specifications

Large package, 48 tests/kit

3. Storage and Shelf Life

The shelf life of the kit is 9 months stored at -20±5ºC.

Repeated freezing and thawing should be avoided, and the freeze-thaw cycles should not exceed 4. After the reagent is opened, it should be placed at 10ºC-30ºC for no more than 8 hours. Dry ice (or ice pack) is used for transportation to keep the low temperature, and the transportation time should not exceed 4 days.

See the product label for the manufacture date and expiration date of the kit.

4. Intended Use

This kit is used for the quantitative detection of hepatitis C virus (HCV) RNA in human serum or plasma specimens.

HCV is a globally infectious pathogen that can cause acute and chronic viral hepatitis. Long-term chronic hepatitis C can result in chronic inflammation, necrosis and fibrosis of the liver, moreover, cirrhosis and even hepatocellular carcinoma can develop in some patients which is extremely harmful to the health and life of patients, and has become a serious social and public health problem. Hepatitis C infection is distributed worldwide. According to the World Health Organization, the global HCV infection rate is about 3%, and there are approximately 35,000 new cases of hepatitis C each year. HCV is a positive-sense single-stranded RNA virus of the family *Flaviviridae*, whose genome consists of around 9,400 nucleotides and is susceptible to mutation. HCV species is classified into 6 genotypes with several subtypes within each genotype. The viral genome copies can reach to 10^5-10^7 copies/mL in plasma or serum of patients with acute hepatitis C. The HCV RNA levels of patients with chronic hepatitis C vary greatly among individuals, ranging from 5×10^4 to 5×10^6 copies/mL, however, the HCV RNA level in the blood of the same patient is relatively stable.

The test results of this kit are for clinical reference only and cannot be used solely as the basis for diagnosis or exclusion of cases. This kit is not intended for use as a screening test for the presence of HCV in blood or blood products.

5. Test Principle

Based on real-time fluorescent PCR technique, this kit takes the relatively conserved region of the HCV gene coding region as the target to design specific primers and fluorescent probe and performs one-step RT-PCR amplification for HCV RNA quantitative testing in serum or plasma specimens. In addition, this kit contains internal control for monitoring the whole process of nucleic acid extraction, amplification and detection to reduce the occurrence of false negative results.

Use the recommended nucleic acid extraction reagent to process the clinical specimen and extract the nucleic acid. The PCR detection reagents provided in the kit is used to prepare the PCR reaction mixture. The extracted nucleic acid is added into the PCR reaction mixture and the fluorescence quantitative PCR instrument is used for one-step RT-PCR amplification, and the fluorescence signal is detected. The real-time amplification curve is automatically drawn by the instrument software system, and the quantitative detection of unknown specimen is conducted according to the threshold cycle value (Ct value).

6. Main Components

Component name		Specification	Quantity	Main constituents
Quality control and positive quantitative references	Negative Control	600 μL/tube	1	Negative plasma
	HCV High Positive Control	600 μL/tube	1	Virus-like particles containing HCV target fragment
	HCV Borderline Positive Control	600 μL/tube	1	Virus-like particles containing HCV target fragment
	HCV Positive Quantitative Reference 1 (1.0×10^6 IU/mL)	600 μL/tube	1	Virus-like particles containing HCV target fragment
	HCV Positive Quantitative Reference 2 (1.0×10^5 IU/mL)	600 μL/tube	1	Virus-like particles containing HCV target fragment
	HCV Positive Quantitative Reference 3 (1.0×10^4 IU/mL)	600 μL/tube	1	Virus-like particles containing HCV target fragment
	HCV Positive Quantitative Reference 4 (1.0×10^3 IU/mL)	600 μL/tube	1	Virus-like particles containing HCV target fragment
PCR detection reagents	HCV Internal Control	250 μL/tube	1	Virus-like particles containing internal control fragment, and stabilizer
	HCV Reagent A	96 μL/tube	1	HCV specific primers and probe

	HCV Reagent B	144 μL/tube	1	Hot-start Taq polymerase, RNasin, c-MMLV Reverse Transcriptase
	HCV Reagent C	720 μL/tube	1	$MgCl_2$, Tris-HCl Buffer, Brij-58

Note: the components above in different batches of kits cannot be used interchangeably.

7. Applicable Instrument

ABI Prism 7500

8. Materials and Instruments Required but Not Provided

When using the kit, materials and instruments required but not provided by manufacturer should be supplied by user, including but not limited to lab coat, disposable gloves and mask (powder-free), pipette (adjustable), sterile pipette tips with filters, sterile centrifuge tubes, sterile PCR reaction tubes, thermostatic heater, timer, real-time PCR amplification instrument, desktop high speed centrifuge, and nucleic acid extraction or purification reagents (Nucleic Acid Isolation or Purification Reagent(Cat.# DA0860 ~ 0863, YSXB No. 20181277) , Nucleic Acid Isolation or Purification Reagent (Cat.# DA0620 ~ 0626, YSXB No. 20170583) produced by Daan Gene Co., Ltd. are recommended).

9. Specimen Requirements

9.1 Applicable specimen type: serum or plasma.

9.2 Specimen collection:

9.2.1 Serum: use a disposable sterile syringe to draw 2 mL of venous blood from the subject, inject it into a sterile dry glass tube, and place it at room temperature (15ºC-25ºC) for 30-60 minutes. The blood specimen could be completely agglutinated to separate out serum spontaneously, or the blood specimen could be centrifuged directly in a horizontal centrifuge at 1,500 rpm for 5 minutes, then draw the upper serum and transfer it to a 1.5 mL sterile centrifuge tube.

9.2.2 Plasma: use a disposable sterile syringe to draw 2 mL of venous blood from the subject, inject it into a glass tube containing EDTA-2Na (disodium ethylenediaminetetraacetate) or sodium citrate anticoagulant, and then hold the glass tube upright, gently invert 180 degrees and back for 5-10 times to mix the anticoagulant and venous blood thoroughly. After 5-10 minutes, the plasma can be separated out, and then transfer it to a 1.5 mL sterile centrifuge tube.

9.3 Specimen preservation and transportation: the collected specimens should be tested immediately or stored at -70°C for long-term storage, or it can also be stored at -20°C for test and the storage

period is 3 months. Repeated freezing and thawing of the specimens should be avoided. Specimen should be transported at 0°C.

10. Test Method

10.1 Specimen processing and nucleic acid extraction (specimen processing area)

The nucleic acid extraction or purification reagents produced by Daan Gene Co., Ltd. can be used for specimen processing and nucleic acid extraction. The internal control is involved in the extraction process. If the method of using the internal control is not specified in the extraction kit, nucleic acid extraction can be carried out after adding internal control to the specimen according to the volume proportion of specimen: internal control = 50 : 1.

The Negative Control and Positive Control in this kit need to be extracted for environmental monitoring and quality control of the PCR detection reagents.

10.2 PCR reagent preparation (reagent preparation area)

Take the HCV Reagent A, HCV Reagent B, and HCV Reagent C from the kit. After thawing them at room temperature, vortex them well, and centrifuge at 8,000 rpm for a few seconds before use.

Take N PCR reaction tubes (N = number of specimens to be tested + Negative Control + HCV High Positive Control + HCV Borderline Positive Control + 4 tubes of HCV Positive Quantitative References). A single-reaction amplification mixture is prepared as follows:

Component	HCV Reagent A	HCV Reagent B	HCV Reagent C	Total volume
Dosage	2 μL	3 μL	15 μL	20 μL

After thoroughly mixing the components, centrifuge it for a short time to make all the liquid on the tube wall fall to the bottom of the tube, and then aliquot 20 μL of the amplification mixture into each PCR tube.

10.3 Sample loading (specimen preparation area)

Add 40 μL of the specimen RNA extracted to be tested, HCV Negative Control, HCV High Positive Control, HCV Borderline Positive Control, and Positive Quantitative References into the PCR reaction tubes respectively by pipette tips with filter, cap the tubes tightly, and centrifuge them at 8,000 rpm for a few seconds and then transfer them to the amplification detection area.

10.4 PCR amplification (amplification detection area)

10.4.1 Place the PCR reaction tubes in the sample sink of the instrument.

10.4.2 **Setting of ABI Prism 7500 Instrument**

a. Open the "Setup" window, set the Negative Control (NTC), Positive Control, unknown specimen (Unknown), and Positive Quantitative References (Standard) in the corresponding order of the samples, and set the sample name in the column of "Sample Name". The probe detection modes are set as: Reporter Dye1: FAM, Quencher Dye1: TAMRA, Reporter Dye2: VIC, Quencher Dye2: none, Passive Reference: NONE.

b. Open the "Instrument" window and set the cycle conditions as follows:

50°C for 15 minutes, 1 cycle;

95°C for 15 minutes, 1 cycle;

From 94°C for 15 seconds to 55°C for 45 seconds (fluorescence collecting), 45 cycles.

After setting, save the file and run the program.

10.5 **Analysis of test results**

After amplification, test results are automatically saved, and the "Start Value", "End Value", and "Threshold Value" of the "Baseline" can be adjusted according to the analyzed image (the user can adjust it according to the actual situation. The "Start Value" can be set between 3-15, and the "End Value" can be set between 5-20. Set the "Threshold Value" in the "Log" spectrum window so that the threshold line is in the exponential phase of the amplification curve, and the amplification curve of the Negative Control is flat or lower than the threshold line), click "Analysis" to automatically obtain the analysis results, check the results on the "Report" interface, and record the unknown specimen value "C".

10.6 **Quality control**

Negative Control: the amplification curve of FAM detection channel has no logarithmic growth phase, and the amplification curve of VIC detection channel has a logarithmic growth phase.

HCV Positive Controls: the amplification curve of FAM detection channel has a significant logarithmic growth phase, showing a typical S-shaped amplification curve. The quantitative value of HCV High Positive Control ranges from 1.0×10^5 to 5.0×10^6 IU/mL, and the quantitative value of HCV Borderline Positive Control ranges from 1.0×10^2 to 1.0×10^4 IU/mL.

HCV Positive Quantitative References: the amplification curve of FAM detection channel has a significant logarithmic growth phase, showing a typical S-shaped amplification curve, and the Ct value <40, $R^2 \geq 0.97$.

The requirements above must be met at the same time in the same test, otherwise, the test is invalid and needs to be carried out again.

10.7 **Judgement of test results**

10.7.1 If the amplification curve of FAM detection channel has no logarithmic growth phase and the amplification curve of VIC detection channel has a logarithmic growth phase, the HCV RNA concentration of the tested specimen is judged to be lower than the detection sensitivity of the kit.

10.7.2 If the amplification curve of FAM detection channel has a significant logarithmic growth phase and Ct value <45, determine the results according to the following method:

a. If the test result of specimen satisfies 5.00E+001≤ C ≤1.00E+008, then the HCV RNA concentration of the specimen = C IU/mL;

b. If the test result of specimen satisfies C >1.00E+008, then the HCV RNA concentration of the specimen >1 × 10^8 IU/mL. If accurate quantitative result is needed, dilute the specimen with Negative Control to the linear range before testing. Then the HCV RNA concentration of the specimen = (C × dilution ratio) IU/mL;

c. If the test result of specimen satisfies 2.00E+001≤ C <5.00E+001, then the HCV RNA concentration of the specimen is for reference only;

d. If the test result of specimen satisfies C <2.00E+001, meanwhile the amplification curve of VIC detection channel has a logarithmic growth phase, then the HCV RNA concentration of the specimen is lower than the detection sensitivity of the kit.

11. Cut-off Value

According to the test results of clinical specimens, the cut-off value of this kit is determined: the Ct value is 45.

12. Interpretation of Test Results

12.1 The Negative Control, HCV High Positive Control, and HCV Borderline Positive Control should be tested for each test. Only when the quality control results meet the requirements of *10.6* ***Quality control,*** the test results can be judged.

12.2 Criteria for positive result: the amplification curves of FAM and VIC detection channel both have a logarithmic growth phase. Or the amplification curve of FAM detection channel has a logarithmic growth phase and the amplification curve of VIC detection channel has no logarithmic growth phase.

12.3 Criteria for negative result: the amplification curve of FAM detection channel has no significant logarithmic growth phase and the amplification curve of VIC detection channel has a logarithmic growth phase.

12.4 Following report format is recommended:

Report format on negative result: HCV RNA is not detected in the specimen, and its concentration is lower than the detection sensitivity of the kit;

Report format on positive result:

a. If the test result of the specimen satisfies 5.00E+001 ≤ C ≤1.00E+008, the report format is: HCV RNA is detected in the specimen, with a concentration of C IU/mL.

b. If the test result of specimen satisfies C >1.00E+008, the report format is: HCV RNA is detected in the specimen, with a concentration more than 1×10^8 IU/mL; if the detection is performed after dilution, the report format is: HCV RNA is detected in the specimen, with a concentration of (C × dilution ratio) IU/mL.

c. If the test result of specimen satisfies 2.00E+001≤ C <5.00E+001, the report format is: HCV RNA load is fairly low in the specimen, and the measured value is for reference only.

d. If the test result of specimen satisfies C <2.00E+001, meanwhile the amplification curve of VIC detection channel has a logarithmic growth phase, the report format is: HCV RNA concentration in the specimen is lower than the detection limit of the kit; if the amplification curve of internal control detection channel has no logarithmic growth phase or shows no Ct value, the test result of the specimen is invalid. Find out and eliminate the cause, and the specimen should be tested again (If the result is still invalid, please contact the manufacturer).

12.5 The test result is for clinical reference only. For a confirmation of the case, please combine clinical symptoms and other test procedures to make a definite diagnosis.

13. Limitation of the Test Procedure

13.1 The specimen test result is related to specimen collection, processing, transportation, and preservation.

13.2 If cross contamination is not well controlled during specimen processing, false positive result may occur.

13.3 Gene mutation of virus in an epidemic may cause false negative result.

13.4 Since different extraction methods adopt different principles, there are some differences in the nucleic acid extraction efficiency when a specimen is treated by different nucleic acid extraction reagents, and users can select the nucleic acid extraction reagent according to specific requirements.

13.5 The test result is for clinical reference only. For a confirmation of the case, please combine clinical symptoms and other test procedures to make a definite diagnosis.

14. Performance Characteristics

14.1 The sensitivity of the kit is 20 IU/mL, and the linear range is from 50 to 1.0×10^8 IU/mL.

14.2 Analytical specificity:

a. Cross-reactivity

The kit is free of cross reaction with the viruses that infect the same infection site or lead to similar infection symptoms, or other pathogens (including family *Flaviviridae*, such as West Nile virus etc., dengue fever virus (DV), cytomegalovirus (CMV), EB virus (EBV), human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), hepatitis B virus (HBV), hepatitis A virus (HAV), *Treponema pallidum* (TP), human herpes virus type 6 (HSV-6), herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), influenza A virus, *Staphylococcus aureus*, and *Candida albicans*).

b. Interference study

Endogenous interfering substances: The test result of the kit on the HCV specimen is not interfered by bilirubin with concentration ≤ 30 mg/dL, triglyceride with concentration ≤ 3,000 mg/dL, hemoglobin with concentration ≤ 28 g/dL, or albumin with concentration ≤ 6 g/dL.

Exogenous interfering substances: The test result is not interfered by common interferon IFNα with concentration ≤ 14.2 μg/mL, complex IFN with concentration ≤ 14.2 μg/ml, polyethylene glycol interferon α with concentration ≤ 14 ± 2.5 ng/ml, ribavirin with concentration ≤ 1-2mg/L, and

granulocyte stimulating factor (GMCSF) with concentration ≤ 21.6 ng/ml.

14.3 The CV% of the logarithm of the quantitative detection value of intra-batch and inter-batch precision is less than 5%.

15. Warnings and Precautions

15.1 For in vitro diagnostic use only. Please read the *Instructions for Use* carefully before test.

15.2 In order to avoid any potential biohazards in the specimen, the tested specimen should be regarded as infectious, and contacting with human skin and mucosa shall be avoided. It is recommended that the specimen should be processed in a biosafety cabinet that can prevent aerosol outflow. Put the test tubes and pipette tips used in the specimen preparation area into a container with disinfectant, and sterilize them together with the wastes before disposal. Specimen handling and processing must meet the requirements of relevant local laws and regulations.

15.3 The components in the kit should be used within the shelf life. False result may be caused without using the components provided by the kit.

15.4 Laboratory management should be strictly in accordance with regulations on management of PCR gene amplification laboratories. Test operator must receive professional training. Test process should be carried out strictly in separate areas (reagent preparation area, specimen preparation area, and amplification detection area). All consumables should be sterilized for single use. Dedicated instruments and equipment should be used in each stage of the test process. And supplies in different stages and areas should not be used interchangeably.

15.5 Use autoclaved disposable centrifuge tubes and pipette tips or purchase DNase-free/RNase-free centrifuge tubes and pipette tips.

15.6 The PCR reagents should be completely thawed before use, and used after centrifuge at 8,000 rpm for several seconds, but repeated freezing and thawing should be avoided.

15.7 After nucleic acid extraction of the specimen, it is recommended to proceed to next step immediately, otherwise store the nucleic acid at -20°C for later use (within 24 hours).

15.8 If cross contamination is not well controlled during specimen processing, false positive result may occur.

15.9 Quality control procedures shall be performed for each test.

15.10 After the test, the worktable and pipette shall be treated with 10% hypochlorous acid or 75% alcohol first and then exposed in ultraviolet light for 20-30 minutes.

15.11 All positive quality controls in the kit and test specimens should be considered as if infectious, and should be handled and processed in accordance with your local regulations and laws.

16. References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-2128.

2. Strassl R, Rutter K, Stättermayer AF, Beinhardt S, Kammer M, Hofer H, Ferenci P, Popow-Kraupp T. Real-Time PCR Assays for the Quantification of HCV RNA:Concordance, Discrepancies and Implications for Response Guided Therapy. PLoS One. 2015 Aug 14;10(8):e0135963.

17. Basic Information

Manufacturer: Daan Gene Co., Ltd.

Address: No.19, Xiangshan Road, Science Park, High & New Technology Development District, Guangzhou, Guangdong, P. R. China.

Contact information:

Fax: +86-20-32068820 Tel: +86-8008304008 / +86-20-32290789

Post code: 510665 Website: http://www.daangene.com

Production address: No.19, Xiangshan Road, Science Park, High & New Technology Development District, Guangzhou, Guangdong, P. R. China; No.6, Lizhishan Road, Science Park, High & New Technology Development District, Guangzhou, Guangdong, P. R. China.

18. Reference Standards

EN ISO 15223-1:2016 Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements

EN ISO 18113-1:2011 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements

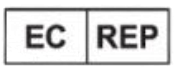
EN ISO 18113-2:2011 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use

19. European Authorised Representative

MDSS GmbH

Address: Schiffgraben 41, 30175 Hannover, Germany

20. Explanation of Symbols

	Use-by date
	Batch code
	Date of manufacture
	Manufacturer
	Catalogue number
	Temperature limit
	Contains sufficient for <n> tests
	In vitro diagnostic medical device
	Do not re-use
	CE marking
	Caution
	European Authorised Representative
	Consult Instructions for Use