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Quantitative Diagnostic Kit for Hepatitis C virus (HCV) RNA

Product Number: DTL0405

Shipping and Storage

1. Store below 30℃. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used the all components should be stored at (-20±5)℃. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. It is not recommended to repeat the freeze-thaw cycle more than 7 times.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
HCV RT-PCR Master Mix	Lyophilized powder ×1 Bottle
HCV quantitative standard substance 1	Lyophilized powder ×1 Tube
HCV quantitative standard substance 2	Lyophilized powder ×1 Tube
HCV quantitative standard substance 3	Lyophilized powder ×1 Tube
HCV quantitative standard substance 4	Lyophilized powder ×1 Tube
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

Note: Do not mix reagents from different batches.

The reaction system is lyophilized powder that contains all components required for fluorescence PCR, including Taq enzyme, reverse transcriptase, primers, probes, dNTPs, and Mg^{2+}.

Description

This kit uses multiple fluorescent PCR detection technology to achieve quantitative detection of HCV RNA in the sample to be tested by using a pair of specific primers and a specific fluorescent probe for HCV. The kit is provided with an Internal Control, which can monitor whether there is PCR inhibitor in the sample to be tested by detecting whether the internal control is normal or not, so as to avoid false negative PCR.

Application

This kit is suitable for the quantitative detection of Hepatitis C virus nucleic acid (HCV RNA) in human serum or plasma samples in vitro, and the test results are used for the auxiliary diagnosis of Hepatitis C. The test results are not the only indicators for the evaluation of the patient's condition, and the condition must be comprehensively analyzed in combination with the patient's clinical manifestations and other laboratory tests.

Hepatitis C is a major blood-borne disease. Chronic infection of Hepatitis C virus can lead to chronic inflammation, necrosis and fibrosis of the liver. Some patients may develop liver cirrhosis or even hepatocellular carcinoma, which is extremely harmful to the health and life of patients. , has become a serious social and public health problem. HCV belongs to the family flaviviridae, and its genome is single-stranded positive-stranded RNA, which is easily mutated. Currently, it can be divided into 6 genotypes and more than 50 different subtypes. HCV genotypes 1b and 2a are relatively common in my country, among which type 1b is the main type, types 1a, 2b and 3b have been reported in some areas, type 6 is mainly found in Hong Kong and Macau, and this genotype can also be seen in the southern border provinces. HCV indicators include anti-HCV and HCV RNA. Although some patients with acute Hepatitis C can recover by themselves, active treatment should be given to all patients with acute Hepatitis C, because the curative effect in the acute phase is good. In the treatment of chronic Hepatitis C, only interferon is recognized as effective at home and

abroad. It should also be treated early. In the recovery period of acute Hepatitis C, more than 70% of patients who choose interferon can be cured, which can make chronic Hepatitis C. The number of patients is greatly reduced. If the patient delays the treatment time in the acute phase, about 60% of the infected patients will develop chronic Hepatitis C, and the possibility of self-healing in the future is very small, so the etiological diagnosis should be carried out as soon as possible.

Applicable instruments

Real-time fluorescence PCR instrument with FAM,VIC channels.

Specimen collection

1. Applicable sample type: Serum specimens.
2. Sample collection: Use a sterile syringe to draw 2mL of the subject's venous blood and inject it into a sterile centrifuge tube. Leave it at room temperature for no more than 4 hours. Centrifuge at 2000rpm for 5 minutes. Take the upper serum (be careful not to bring in red blood cells) and transfer it to another sterile centrifuge tube for use.
3. Sample storage and transportation: The collection or processing sample should not exceed 24 hours under the conditions of 2℃ ~ 8℃. If long-term preservation is needed, it should be stored below -70℃, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the HCV RT-PCR Master Mix, open each bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 60 seconds until the liquid is clear and transparent. Subpackage it into PCR reaction tubes according to 20μL/ tube.

2. Nucleic acid extraction:

This kit is not included for Nucleic Acid(NA) extraction reagent.

Commercially available extraction kits that have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted RNA is not used immediately, it should be stored below -20℃. For long-term storage, it should be stored below -80℃ and avoid repeated freezing and thawing.

Note: The Negative Control and the Positive Control does not require nucleic acid extraction.

The HCV quantitative standard substances 1~4 needs to be redissolved with 100μL of Redissolved Diluent and mixed well before use separately.

3. Add sample:

The correspond substances were added to that above PCR reaction tubes according to the following table:

Type	Add sample description
Testing Sample	Add 5μL of the extract prepared in step 2 to the reaction tube, and close the tube cover.
Negative Control/ Positive Control	Add 5μL of negative control and positive control to the reaction tube, and cover the tube tightly.
HCV quantitative standard substances 1,2,3,4	Add 5μL of HCV quantitative standard substances 1,2,3,4 respectively, to the PCR reaction tube, and cover the tube tightly.

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be centrifuged for 15s on a palm centrifuge and then delivery to the nucleic acid amplification region. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR amplification:

Place the reaction tube in the automatic fluorescent PCR instrument, set the Negative control, Positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding

sample name.

Select FAM channel to detect HCV RNA, select VIC channel to detect the IC. Set the Reaction Volume per Well to 25μL.

(Note: For ABI series instruments, select 'None' under 'Quencher', and select 'None' as the dye to use as the passive reference.)

Recommended reaction program setting:

Step	Cycles	Temperature	Time	Collect fluorescence signal
1	1 cycle	50℃	10min	No
2	1 cycle	95℃	2min	No
3	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis:

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3~15, the End value can be set at 5~20, the amplification curve of the negative control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis, make the parameters meet the requirements in the following '6.Quality control', and then go to the Plate window to record the Ct value.

6. Quality control

Negative control: FAM and VIC detection channels have no amplification curves,in the Reports interface, the Ct column displays 'Undet'.

Positive control:The FAM and VIC channels have obvious S-shaped amplification curves, and the Ct value is ≤32.00. HCV quantitative standard substances 1~4:FAM channel has obvious amplification curve, and the standard curve correlation coefficient $|r| \geq 0.98$.

The above requirements must be met at the same time in the same experiment. Otherwise this experiment is invalid and needs to be repeated.

Positive Judgment Value

Through the study of the reference value, the minimum detection limit of this kit for HCV was determined to be 500 copies/mL, and the Ct positive judgment value of VIC was 32.00.

Explanation of Test Result

1. If the Ct value of the VIC channel of the test sample is >35.00, the test result of the sample is invalid, and the cause should be found and eliminated. Samples need to be recollected and the experiment repeated.
2. For samples with measured value $<5.00\times10^2$ copies/mL or Ct value was 'Undet', and the Internal Control detection is positive and Ct value ≤32.00, it was reported as lower than the detection limit of the kit. If the Internal Control is abnormal, the test result of this sample is invalid, and the test is repeated for this sample.
3. For samples with measured values between 5.00×10^2 copies/mL~1.00×10^8 copies/mL, report the corresponding determination results.
4. For samples with measured value $>1.00\times10^8$ copies/mL, the Internal Control detection is positive and Ct value ≤32.00, It is recommended to re-measure after diluting with 1×TE, calibrate the results according to the dilution factor, report the calibrated value, and indicate "reportable concentration",and the measurement results are for reference only.

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. If cross-contamination is not controlled during the sample extraction process, false positive results will occur.
3. Contamination from the Positive Control or amplified products (amplicons) can lead to false positive results.
4. Genetic mutations or rearrangements in the target sequence may lead to false negative results.

5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Degradation of reagent performance due to improper transportation, storage, or inaccurate preparation may lead to false negative results or inaccurate quantification.
7. The results of this test are for reference only. Clinical diagnosis should be based on a combination of clinical symptoms, other laboratory findings, and epidemiological data.

Performance Parameters

1. Minimum detection limit: The minimum detection limit of this reagent is 5.00×10^2 copies/mL.
2. Linear range: 5.00×10^2 copies/mL~1.00×10^8 copies/mL.
3. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) value of detected concentration logarithm is ⩽5.00%.
4. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise reference is 100%, and the compliance rate of positive reference products is 100%.
5. Analysis of Specificity: Cross-reaction: There is no cross-reaction with cytomegalovirus, Hepatitis A virus, Hepatitis B virus, Adenovirus, HSV-1, HSV-2, VZV, NG, HHV-6, Staphylococcus aureus, Candida albicans; Interfering substances: common interfering substances such as mucin, blood, commonly used drugs for patients with common respiratory symptoms epinephrine, dexamethasone, sulfur, gold, menthol, zanamivir, mupirocin, tobramycin are under test When the concentration in the sample is 0.1mg/mL, 10%, 100pg/mL, 100pg/mL, 4.5mg/mL, 4.5mg/mL, 5mg/mL, 3mg/mL, 10mg/mL, 0.3mg/mL respectively do not interfere with the test results of the kit.

Note

1. Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and carry out quality control for each experiment.
3. The reaction solution should be stored away from light.
4. Try to avoid bubbles in the reaction, and the tube cover needs to be tight.
5. Use disposable heads, disposable gloves and special work clothes in each district.
6. Sample processing, reagent preparation, and samples need to be performed in different areas to avoid cross -pollution.
7. After the experiment is completed, use 10% hypochlorite or 75% alcohol or ultraviolet light to treat the workbench and pipette.
8. All items in the kit should be treated as pollutants and processed in accordance with the "Biological Safety General of Microbiological Biomedical Laboratory".