MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Diagnostic Kit for Hepatitis C Virus (HCV) RNA

Product Number: DTL0404

Shipping and Storage

1. Store below 30°C. 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)°C. 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
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

1. Do not mix reagents from different batches.
2. 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 qualitative 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 qualitative 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 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°C, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the HCV RT-PCR Master Mix, open the 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.

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 individually to the 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. RT-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 'Quality control', and then go to the Plate window to record the Ct value.

6. **Quality control**

Negative control: FAM,VIC detection channels has no obvious amplification curve.

Positive control: FAM,VIC detection channels has an obvious amplification curve, and the Ct value ≤32.00.

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

Explanation of Test Result

Positive: The Ct value of the FAM channel is ≤40.00, and the curve has an obvious exponential growth curve, and the VIC channel has or does not have an amplification curve.

Negative: The Ct value of the FAM channel is >40.00 or there is no Ct value,and the Ct value of the Internal Control is ≤40.00.

If the FAM channel amplification curve of the test sample has no logarithmic growth phase and the VIC channel Ct value is >35.00, the test result of the sample is invalid and the cause should be found and eliminated. Collect samples again and repeat the experiment.

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. There is no control of cross -pollution during the sample extraction process, and false positive results will occur.
3. Positive control and leakage of amplification products can lead to false positive results.
4. The genetic mutations and reorganizations during epidemics can lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Reagent transportation, improper preservation, or inaccurate reagent preparation reagent detection performance decreases, and the results of false negative or quantitative detection occur.
7. The results of this test are for reference only. If the diagnosis must be confirmed, please combine clinical symptoms and other test methods.

Performance Parameters

1. Limit of Detection: The minimum detection limit of this reagent is 500 copies/mL.
2. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) of the Ct value of the pathogen detection channel is ≤5.00%.
3. 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%.

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% hypochloride 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".