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Hepatitis B Virus/Hepatitis C Virus/Human Immunodeficiency Virus Type 1 (HBV/HCV/HIV-1) Triple Probe-Based Quantitative RT-PCR Kit

Product Number: DTK568

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of one year. Positive controls need to be placed separately and should not contaminate other reagents.

Component

Component	Specifications
2 × OneStep Probe Mix	1250μL
OneStep Probe Enzyme Mix	200μL
DEPC-H_2O	1mL×2
Triple RT qPCR Primer Mix	2300μL
Triple RT qPCR probe mixture	120μL
Triple RT qPCR positive control (1 × 10E8 copies/μL)	60μL

Description

This kit can quickly detect three viruses in one reaction: hepatitis B virus, hepatitis C virus, and human immunodeficiency virus type 1.

Application

Developed based on the principle of probe based fluorescence quantitative RT-PCR, it has the following characteristics:

1. Ready to use, users only need to provide nucleic acid (including DNA and RNA) templates.
2. Provide positive controls for three viruses to distinguish false negative samples.
3. Fast response and high sensitivity.
4. High sensitivity and strong specificity to all individual pathogens.
5. Can be used for high-throughput detection.
6. This kit is sufficient for 96 times of probe based fluorescence quantitative PCR in a 25μL system and can only be used for scientific research.

Protocol

1. **Nucleic acid extraction (sample preparation area)**

   Extract and purify nucleic acid samples using a self selected method. This kit is compatible with most nucleic acid extraction kits on the market. We recommend using our company's virus genome DNA/RNA extraction kit to extract nucleic acids.

2. **Dilute standard curve sample (sample preparation area)**

   (Due to the high concentration of positive control, the following dilution operations must be performed in a separate area to avoid contaminating the sample or other components of this kit.)

   2.1. Mark 6 centrifuge tubes, namely 7, 6, 5, 4, 3, 2.

   2.2. Add 45μL of fluorescent template diluent separately using a core gun tip (preferably using a core gun tip, the same below).

   2.3. Add 5μL of 1 × 10E8 copy/μL positive control (provided by the reagent kit) to tube 7, shake thoroughly for 1 minute, and

obtain 1 × 10E7 copy/μL standard curve sample. Put it on ice for later use.

2.4. Change the gun head and add 5μL of 1 × 10E7 copy/μL positive control (obtained from the previous dilution) to tube 6. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E6 copy/μL. Put it on ice for later use.

2.5. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (obtained from the previous dilution) to tube 5. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E5 copy/μL. Put it on ice for later use.

2.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use. If no standard curve is required, dilute the positive control to 1 × 10E5 copies/μL.

3. Reagent Preparation (Reagent Preparation Area)

If there are N samples to be tested, prepare N+2 qPCR tubes (N samples to be tested+1 negative control+6 positive control) and add the following components to each qPCR tube.

component	Sample tube N+2	qPCR negative control	qPCR positive control
2 × OneStep Probe Mix	12.5μL each	12.5μL	12.5μL
OneStep Probe Enzyme Mix	2μL each	2μL	2μL
Triple RT qPCR Primer Mix	2.3μL each	2.3μL	2.3μL
Triple RT qPCR probe mixture	1.2μL each	1.2μL	1.2μL
DEPC-H_2O	5μL each	5μL	5μL

Transfer to the sample preparation area.

4. Add Template (Template Add Area)

Add 2μL of template to the qPCR tube, in the order of negative control (DEPC-H_2O), test sample template, triple RT qPCR positive control. Centrifuge for 30 seconds and immediately perform amplification reaction.

5. Amplification reaction (amplification and product analysis area)

Place the qPCR tube in the corresponding position of the qPCR amplification instrument sample slot for amplification. The amplification procedure is as follows:

Step	Temperature	Time
Reverse transcription	50°C	15min
Pre denaturation	95°C	3min
qPCR reaction	95°C	30sec
(40 cycles)	60°C	1min
Channel	Simultaneously select FAM/HEX/Cy5 channels to collect fluorescence signals	

The selection of fluorescent and quenching groups is shown in the following table:

Target	Fluorophores	Quenching group
Hepatitis B virus	FAM	None
Hepatitis C virus	HEX	None
Human Immunodeficiency Virus Type 1	Cy5	None

6. Result analysis

6.1. If creating a standard curve, plot the standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of DNA/RNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and calculate its concentration.

6.2. If no standard curve has been created, the results shall be judged according to the following criteria:

Positive control result: Ct value<30, with significant exponential growth, showing a typical S-shaped curve.

Negative control result: Ct value>38 or no Ct value, no significant exponential growth period or plateau period.

Sample testing results: Ct value<35, with a significant exponential increase, indicating the detection of mouse parvovirus in the sample, and the result is positive; A Ct value greater than 38 or no Ct value indicates that no mouse parvovirus was detected in the sample, and the result is negative; If the Ct value is within the range of 35-38, the sample should be retested. If the Ct value of the repeated experiment is still within the range of 35-38 and there is a significant exponential

increase, it is judged as positive. Otherwise, it is judged as negative.