MEBEP BIOSCIENCE

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Human Immunodeficiency Virus Type 1 (HIV-1) Universal Probe-Based Quantitative RT-PCR Kit

Product Number: DTK566

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of 12 months.

Component

Component	Specification	Nine hole box packaging
Probe based qRT-PCR buffer	500μL	White cover
Probe based qRT-PCR enzyme mixture	100μL	Red cover
Fluorescent PCR specific template diluent	1mL	Green cover
HIV-1 universal probe method qRT-PCR primer probe mixture	150μL	Brown cover
HIV-1 universal probe qRT-PCR positive control (1 × 10E7 copy/μL)	50μL	Yellow cover

Description

Human Immunodeficiency Virus; abbr: HIV, namely AIDS (Acquired Immunodeficiency Syndrome) virus, is a virus that causes defects in the human immune system. In 1981, the human immunodeficiency virus was first discovered in the United States. It is a lentivirus that infects cells of the human immune system and belongs to the category of retroviruses. The current global epidemic is mainly HIV-1.

Application

This product is a specialized kit developed based on probe based fluorescence quantitative RT-PCR technology for the detection of universal human immunodeficiency virus type I. It has the following characteristics:

1. Ready to use, users only need to provide sample RNA templates.
2. Primers and probes have been optimized for high analytical sensitivity.
3. Provide a positive control to distinguish false negative samples.
4. High specificity, primers are designed based on the highly conserved region of human immunodeficiency virus type I and will not cross react with RNA of other viruses.
5. It can be used for both qualitative and quantitative testing. When used for quantitative detection, the linear range should be at least 5 orders of magnitude.
6. This product is sufficient for 50 probe based fluorescence quantitative RT-PCR reactions in a 20μL system.
7. This product can only be used for scientific research.

Specimen collection

Sample RNA, ultrapure water.

Protocol

1. Dilute the standard curve sample (taking the 10 fold dilution of 6 copies/μL of 10E1-10E6 as an example)

Due to the high concentration of the standard substance, the following dilution operations must be carried out in a separate area and must not contaminate the sample or other components of this kit. To increase product stability and avoid the spread of infectious pathogens, this product does not provide live samples as positive controls, only non infectious DNA fragments are

provided as positive controls.

1.1. Mark 6 centrifuge tubes, namely 6, 5, 4, 3, 2, and 1.

1.2. Add 45μL of fluorescent RT-PCR template dilution solution using a core gun tip, preferably with a core gun tip.

1.3. Add 5μL of 1 × 10E7 copy/μL positive control (provided by the reagent kit) to tube 6, shake thoroughly for 1 minute, and obtain 1 × 10E6 copy/μL standard curve sample. Put it on ice for later use.

1.4. 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.

1.5. Change the gun head and add 5μL of 1 × 10E5 copies/μ L positive control (obtained from the previous dilution) to tube 4. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E4 copies/μL. Put it on ice for later use.

1.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use.

2. Preparation of Sample DNA

2.1. If there are N samples, it is best to set N+2 extractions, with the additional being PC (positive control for sample preparation) and NC (negative control for sample preparation). You can take 10μL of 1000 fold dilution of the positive control and add a certain amount of water to make the total volume consistent with the specified volume of the sample to be extracted, and use it as PC. Additionally, use water as NC.

2.2. Purification of RNA samples using self selected methods, this kit is compatible with most RNA extraction kits on the market. We recommend using our company's RNA extraction kit for extraction.

3. Probe qRT-PCR reaction (20μL system, performed in the sample preparation room)

3.1. If quantitative analysis is performed and only one replicate is performed, label N+9 RT-PCR tubes, including N+2 for the N+2 samples obtained in the previous step, 1 for RT-PCR negative control (using water as a template), and 6 for the standard curve. If qualitative analysis is performed and only one repetition is made, label N+4 RT-PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for RT-PCR negative control (using water as a template), and 1 is used for RT-PCR positive control (using the positive control dilution of tube 4 as a template). Below, only quantitative analysis will be used as an example to describe the operational steps.

3.2. Add each component to the labeled tube according to the table below (this table only lists one repetition. The positive control is only set after the sample tube and negative control are set, and the positive control sample should be added after all tubes are covered and stored):

Component	Sample tube N+2	RT-PCR negative control tube	Standard curve sample tube (1-6 tubes)
Probe based qRT-PCR buffer	10μL	10μL	10μL
Probe based qRT-PCR enzyme mixture	2μL	2μL	2μL
HIV-1 universal probe method qRT PCR primer probe mixture	3μL	3μL	3μL
N+2 RNA samples to be tested	5μL	-	-
Self provided ultrapure water	-	5μL	-
Step 6: Standard Curve Sample Dilution Solution (1-6)			5μL (sample 1 to tube 1, sample 2 to tube 2...)

3.3. Cover the machine with the lid and perform RT-PCR according to the following parameters:

Process	Temperature	Time
Reverse transcription	50°C	20 min
Pre denaturation	95°C	10 min
qPCR reaction (45 cycles)	95°C 60°C	15sec 60 sec (Collect fluorescence signals from FAM channels)

4. Data processing

4.1. If this reagent kit is used for quantitative detection, plot a 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 the RNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and then calculate its concentration.

4.2. If this kit is used for qualitative testing and only determines positive or negative, the negative control Ct must be greater than or equal to 40. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct value should be less than or equal to 35. For the test sample, if its Ct is greater than or equal to 40, it is negative; if it is less than or equal to 35, it is positive. If it is between 35-40, repeat once. If the Ct value of the repeated experiment is greater than or equal to 40, it is negative; if it is less than 40, it is positive.