Human Immunodeficiency Virus (HIV) Detection Kit (Real-time PCR)

Instruction for Use (V1.1)

[REF] UBP-S00150H

[Specification] 50 tests/kit

[Research Use Only]

The human immunodeficiency virus nucleic acid assay kit (hereinafter referred to as the Kit) is used for the quantitative detection of human immunodeficiency virus RNA in human serum or plasma samples.

The human immunodeficiency virus (HIV), which lives in a person's blood, can destroy the body's immune system, thus making the body lose its ability to fight off other diseases, causing incurable infections and tumors, and eventually death. HIV can be transmitted through sexual contact, blood and from mother to child.

This kit is suitable for the quantitative detection of HUMAN immunodeficiency virus RNA in serum/plasma samples, and can monitor the HIV level in the blood of human immunodeficiency virus patients, providing an auxiliary means for the detection of human immunodeficiency virus patients; The test results are for research use only, and the final conclusion should be considered in conjunction with other indicators.

This kit should not be used for blood source screening.

[Test Principles]

This kit uses a combination of PCR amplification and fluorescence probe to design specific detection primers and fluorescence probe for the conserved regions of human immunodeficiency virus. The probe was an oligonucleotide labeled with 5 'FAM fluorescence group and 3' BHQ1 quenching group. When the probe was intact, the fluorescence signal emitted by the reporter group was absorbed by the quenched group. During PCR amplification, the 5 '-3' exonuconase activity of Taq enzyme will degrade the probe enzyme and separate the reporting and quenching fluorophore, so that the fluorescence signal can be received by the fluorescence monitoring system. That is, for each AMPLIFIED DNA strand, a fluorescence molecule is formed, and the accumulation of fluorescence signal is fully synchronized with the formation of PCR products. The extracted RNA was reverse transcribed into cDNA, and quantitative fluorescence PCR detection technology was used to detect the RNA in the sample by the change of fluorescence signal in the PCR process and the change of fluorescence signal value of RNA standard amplification. At the same time, exogenous internal reference was added to control the quality of reagent, DNA and operation itself to avoid false negative.

[Kit Contents]

No.	Name	Specification	Description
1	HIV Reaction Solution	1.2mL/vial×1	HIV specific primers, fluorescent probes, internal standard gene primers, fluorescent probe RT-PCR mix, Taq enzyme, etc.
2	HIV Reverse Transcriptase	75 μL/vial×1	Reverse transcriptase, glycerol, etc.
3	HIV Internal Control	500 μL/vial×1	Exogenous internal
4	HIV Positive Control	125 μL/vial×1	Mixture of HIV and internal reference template
t	HIV Blank Control	125 μL/vial×1	RNase-and DNase-free H2O

Noted: The contents in different batches of reagents are not interchangeable within the shelf life.

Reagents required but not provided:

DNA/RNA Extraction Kit (Magnetic beads) from ULTRASSAY.

Self-prepared test materials:

RNase and DNase free Tip, disposable gloves, 1.5mL RNase and DNase free centrifuge tube, 0.2mL fluorescent quantitative PCR8 tubes.

[Storage Conditions and Shelf Life]

This kit should be stored in dark conditions below -18℃ for 9 months (please use within the validity period). After opening, repeated melting times shall not be more than 4 times.

It can be stably stored for up to 5 days under the conditions of -18℃ under dark conditions. Production date and expiration date: see the packing box.

[Applicable Equipment]

Applied Biosystems 7500 Real-Time PCR Systems, BioRad CFX96 Real- Time PCR System, Ultrassay XP96 Real Time qPCR System

[Acceptable Specimens]

1. Sample collection

This kit is suitable for serum or plasma samples. When collecting serum samples, venous blood of the subject is taken. 2mL was extracted with a sterile syringe needle, collected in a sterile centrifuge tube, stored at room temperature for no more than 4 hours, centrifuged at 1600rpm for 20 minutes, extracted serum (do not inhale red blood cells) and transferred to another sterile centrifuge tube for standby. When collecting plasma samples, 2mL venous blood of sterile injection needle was placed in a sterile centrifuge tube containing EDTA as an anticoagulant (heparin was not used for anticoagulation), and stored at room temperature for no more than 4 hours, centrifuged at 1600rpm for 20 minutes at room temperature, separated plasma (do not inhale red blood cells), and transferred to a sterile centrifuge tube for reserve.

2. Sample storage

Samples to be tested should not be stored at 2-8℃ for more than a week; Store at -18℃ for no more than 6 months. The extraction of frozen samples should be carried out after the samples are completely melted and mixed. Repeated freezing and thawing should be avoided, and repeated freezing and thawing should not be more than 5 times.

3. Sample transportation

Use foam box with ice seal for transportation.

[Test Procedures]

1. Sample processing

1.1Take 1.5mL RNase and DNase free centrifuge tube, add 10μ l of HIV internal control to the sample to be tested, and use recommended DNA/RNA extraction kit for subsequent SAMPLE RNA extraction, please strictly follow the instructions for specific steps. The extraction volume was 200μL, and the recommended eluting volume was 80μL.

1.2 RNA samples extracted should be immediately used for detection or stored below -18℃, and repeated freeze-thaw times should not exceed 4 times.

2. Preparation of reagents for amplification

Take out the HIV reaction solution from the kit, wait for it to dissolve, oscillate and mix, instantaneous centrifugation; The reverse transcriptase was removed and briefly centrifuged.

Calculate the number of samples to be prepared n (n= number of samples +1 tube of blank control +1 tube of positive control +4 tube of HIV Internal control), and prepare PCR-MIX according to the following table; According to the number of samples n, 25μL PCR-MIX was separately packed into the fluorescent quantitative PCR octuple reaction tubes for each well, and the tube cover was pressed tightly to transfer the PCR-mix to the sample processing area quickly. Immediately cryopreservation with nucleic acid reaction solution and reverse transcriptase at -18℃.

	HIV Reaction Solution (μL)	HIV Reverse Transcriptase (μL)
PCR-Mix	23.5 x N	1.5 x N

3. Add sample

The blank control in the kit were added to the set quintuple fluorescent QUANTITATIVE PCR reaction tubes. The RNA extracted in step 1 and the positive control in the kit were 5μL each. The tube cover was pressed and centrifuged at 2000rpm

for 10sec. The fluorescence quantitative PCR 8-strips tubes were put into the fluorescence PCR machine, and the sequence of sampling was blank control, sample to be tested, HIV positive control.

4. The PCR amplification

4.1 Instrument channel and reaction volume selection:

4.1.1 THE FAM channel (Reporter: FAM, Quencher: None) was selected to detect HIV RNA.

4.1.2 Select the VIC channel (Reporter: VIC, Quencher: None) to detect the internal parameters;

4.1.3 Reference Dye: None was selected; (for ABI series only)

4.1.4 Sample Volume is 30. For specific detection channel Settings, refer to the instructions of each instrument.

4.2 PCR amplification conditions:

Step	No. of Cycle	Temperature	Duration	Collect Fluorescent Signal
1	1	50℃	15 minutes	No
2	1	95℃	10 minutes	No
3	5	95℃	15 seconds	No
		55℃	45 seconds	
4	40	95℃	15 seconds	No
		55℃	45 seconds	Yes

5. Result analysis

5.1 ABI7500//ABI7500Fast Baseline and Threshold setting methods for fluorescence quantitative PCR.

Baseline setting: Set baseline starting point to 3 and end point to 15.

Threshold: Set the threshold for each channel. When setting the threshold line of a channel, first select the detected negative reference, remove the checked automatic threshold line, change the option "□√Auto" to "□Auto", and then manually adjust the threshold line so that the threshold line just exceeds the normal negative reference FAM/ VIC (HEX). The highest point of the channel amplification curve (random noise line) shall be taken.

[Reference Range]

The positive judgment value of FAM channel in this kit was determined to be Ct value ≤38, the negative sample Ct value was 0 or no value, 38<Ct<40 was gray area; Ct value of VIC channel detection ≤35.

[Explanation of Test Results]

A. If the FAM channel has a significant amplification curve and the Ct value is less than or equal to 38, the sample is HIV positive.

B. If there is no amplification curve in FAM channel and significant amplification curve in HEX channel and Ct value ≤35, the sample is HIV negative.

C. If there is a significant amplification curve in the FAM channel and 38<Ct<40, and the Ct value of the VIC (HEX) channel is less than or equal to 35, re-detection is required. If the Ct value of The FAM channel is still 38<Ct<40, and the Ct value of the VIC (HEX) channel is ≤35, it will be reported as positive. If no amplification curve is detected again, the Ct value of THE VIC (HEX) channel is ≤35, the test is negative.

D. If there is no amplification curve in the FAM channel and the Ct value in the VIC (HEX) channel is > 35 or there is no Ct value, it is recommended to re-extract samples for detection.

[Limitations of Test Method]

1. The test results of this kit are for research use only

2. Improper sample collection, transfer, storage and processing may lead to incorrect test results.

3. The contamination of amplified products and cross contamination between samples in nucleic acid extraction are also prone to false positive results. Therefore, the use of PCR requires strict laboratory zoning, laboratory management and quality control measures.

4. This test reagent is limited to the above specified sample types and applicable models.

5. The laboratory should be equipped with equipment and operators in strict accordance with the Specifications for Gene Amplification Laboratories, and the operation should be carried out in strict accordance with the instructions.

6. A negative test does not mean that the patient is not infected. The specific conclusion should be determined in combination with other detection results. The reasons for the negative test results may be ① unreasonable sample collection, transport and processing, excessively low virus drops in samples, ② variation of target sequence of virus detection, ③ other unverified interfering factors such as taking antiviral, ④ or infection caused by other viruses or bacteria. **[Product Performance]**

1. The appearance of the kit is intact, and the liquid component is clear and transparent, without any soluble substance.

2. If this kit is used to test the national negative reference n1-N8 or the enterprise negative quality control n1-N5 for human immunodeficiency virus nucleic acid, the test results should be negative, that is, the conformity rate of the negative reference is 100%.

3. Use kits to test national positive reference p1-P8 or enterprise positive quality control P1-P5 for HUMAN immunodeficiency virus nucleic acid; All the test results should be positive, that is, the conformity rate of positive reference products is 100%.

4. The minimum detection limit of the kit is 100IU/ mL for the reference of the national minimum detection limit of human immunodeficiency virus nucleic acid. The enterprise sensitivity quality control products S1-S5 must be detected.

5. The linear correlation coefficient (R) should be ≥0.98 and the absolute deviation of the test result (LG IU/mL) should not exceed ±0.5 when the kit is used to detect the diluting national reference of HUMAN immunodeficiency virus nucleic acid.

6. The precision test showed that the coefficient of variation (CV) within and between batches calculated by Ct value was less than 5.0% when the kit was used to detect the precision control of human immunodeficiency virus nucleic acid for 10 times.

7. Use this kit to detect other viruses or bacteria samples, such as: human cytomegalo virus, epstein-barr virus, human immunodeficiency virus (HIV), hepatitis b virus, hepatitis virus, syphilis, herpes simplex virus type 1, herpes simplex virus type 2, a/h1n1 flu virus, staphylococcus aureus and candida albicans were negative.

[Precautions]

1. Human immunodeficiency virus (HIV) samples are infectious. The collection, storage, transportation and handling of samples should follow the relevant national management norms and biosafety regulations.

2. The experimental area should be partitioned strictly:

Zone 1: Reagent preparation zone - Prepare reagents needed for amplification;

Zone 2: Sample handling area – tree tment of samples to be tested and reference materials; Zone 3: Detection zone -PCR amplification detection.

3. Articles in each area are for exclusive use, and shall not be used cross-over to avoid pollution; Clean the table immediately after the experiment.

4. All reagents in the kit should be fully melted and evenly oscillated before use, and then centrifuged briefly.

5. The extracted RNA stored at 70℃ should be thawed on ice before adding sample and centrifuged briefly.

6. When adding sample, the sample should fall into the reaction liquid completely, and the sample should not stick to the wall of the tube. After adding sample, the tube cover should be pressed as soon as possible.

7. For negative samples, it is necessary to determine whether the amplification signal of the reference substance is normal, so as to ensure the accurate use of the sample processing process and the test reagent, suppress the appearance of samples, and avoid false negative results; For positive test samples, the amplification signal of internal reference substance can not be considered.

8. Immediately after the amplification, take out the fluorescent quantitative PCR octa-reaction tube and seal it in the

autoclave bag for harmless tree tment.

9. When the reaction liquid with reverse transcriptase added is packaged, bubbles should be avoided as far as possible. Before loading, pay attention to check whether the fluorescent quantitative PCR octuple reaction tubes are tightly covered, so as to avoid the leakage of fluorescent substances to pollute the instrument.

10. The tips used in the experiment should be directly injected into the waste tank containing 84 disinfectant, sterilized together with other discarded items, and then discarded.

11. The workbench and various laboratory articles should be disinfected regularly with 75% alcohol or uv lamp.

12. The centrifuge tube and Tip head used in the experiment must be RNase and DNase free.

13. Centrifuge tubes and Tip heads used in the experiment must be treated harmless.

[References]

[1] Wang J L. epidemiology. People's Medical Publishing House, 2002.

[2] C Drosten,E Seifried,WK Roth. TaqMan 5'-nuclease human immunodeficiency virus type 1 PCR assay with phage-packaged Competitive Internal Control for High-throughput Blood Donor screening. Journal of klinical Microbiology, 2001 39 (12) : 4302-4308.

[3] Kabamba-Mukadi B, Henrivaux P, Ruelle J, Delferriere N, Bodeus M, Goubau P. Human immunodeficiency virus type 1 (HIV-1) proviral DNA load in purified CD4+ cells by LightCycler real-time PCR.BMC Infect Dis. 2005 Mar 21; 5:15.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
			Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
	Lot Number		Reference Number
	Number of Tests		Any warnings and/or precaution to take

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