

Human Immunodeficiency Virus Type 1 (HIV-I) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK348

Shipping and Storage

1. $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$, stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.
2. The collected or processed samples should be stored at $2^{\circ}\text{C}\sim 8^{\circ}\text{C}$ for no more than 24 hours; If long-term storage is required, it should be stored at -70°C or below, with no more than 3 freeze-thaw cycles.

Component

Component	50T
HIV-I reaction solution	500 $\mu\text{L}\times 2$
Enzyme solution	50 μL
HIV-I positive quality control product	250 μL
Negative quality control product	250 μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses a pair of human immunodeficiency virus type 1 specific primers, combined with a specific fluorescent probe, to perform in vitro amplification and detection of human immunodeficiency virus type 1 RNA using one-step fluorescent RT-PCR technology for clinical pathogen diagnosis of suspected infected individuals.

Application

This kit is suitable for detecting human immunodeficiency virus type 1 RNA in blood and other specimens of suspected infected patients, and is suitable for auxiliary diagnosis of human immunodeficiency virus type 1 infection. The test results are for reference only.

Applicable instruments

ABI7500, Agilent MX3000P/3005P, LightCycler, Bio-Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

Specimen collection

2mL of suspected infected patient's venous blood was transferred to EDTA-2Na anticoagulant tube.

Protocol

1. Sample processing (sample processing area)

1.1. Sample pre-processing

Whole blood sample: After blood coagulation, take 100ul of serum and transfer it to a sterilized centrifuge tube.

1.2. Nucleic acid extraction

We recommend using our company's nucleic acid extraction or purification reagents (magnetic bead method or centrifugal column method) for nucleic acid extraction. Please follow the reagent instructions for operation.

2. Reagent preparation (reagent preparation area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1)

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negative control tube+1 positive control tube); For every 10 samples, an additional 1 sample is prepared. The preparation of each test reaction system is shown in the table below:

reagent	HIV-I reaction solution	Enzyme solution
Dosage (sample size N)	19 μ L	1 μ L

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20 μ L per tube.

3. Sample addition (sample processing area)

Take 5 μ L of the nucleic acid, positive control sample, and negative control sample extracted in step 1, and add them to the corresponding reaction tubes. Cover the tubes, mix well, and briefly centrifuge.

4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, and set the reaction system to 25 μ L;

Fluorescence channel selection: Detection channel (Reporter Dye) FAM, Quenching channel (Quencher Dye) NONE, ABI series instruments. Do not select ROX reference fluorescence, select None.

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	50°C	10min	No
2	1 cycle	95°C	2min	No
3	45 cycles	95°C	15sec	No
		60°C	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

After the reaction is completed, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End~value, and Threshold value of the Baseline (~can be adjusted by the user according to the actual situation, the Start value can be set at 3-15, and the End value can be set at 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of the negative quality control product is flat or lower than the threshold line). Click Analyze to automatically obtain the analysis results.

5.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 40 , and the curve shows a significant exponential growth curve;

Negative: The sample test result shows no Ct value and no specific amplification curve.

Suspicious: If the sample test result is $40 < Ct \text{ value} \leq 45$, it is recommended to repeat the test. If the detection channel is still $40 < Ct \text{ value} \leq 45$ and the curve has a clear exponential growth curve, it is judged as positive. Otherwise, it is judged as negative.

Quality control standards

1. Negative quality control product: no specific amplification curve or Ct value display;
2. Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤ 32 ;
3. The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

Limitations of detection methods

1. The results of sample testing are related to the quality of sample collection, processing, transportation, and preservation;
2. Failure to control cross contamination during sample extraction can result in false positive results;
3. Leakage of positive controls and amplification products can lead to false positive results;
4. Genetic mutations and recombination of pathogens during epidemics can lead to false negative results;
5. Different extraction methods have differences in extraction efficiency, which can lead to false negative results;
6. Improper transportation, storage, or preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;
7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.



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Note

1. All operations must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".