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HIV-1 Real Time RT-PCR Kit
User Manual
For In Vitro Diagnostic Use Only

REF SR-0020-02-A+B

IVD

-20 °C

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For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler®480 Instrument

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1. Intended Use

HIV-1 real time RT-PCR kit is used for the detection of HIV-1 in serum or plasma by using real time PCR systems.

Its characteristics:

High sensitivity: lower detection line 10^3 IU/ml； LOQ：2×10^3 ～10^8 IU/ml

（*Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.*）

High specificity: test result will be positive, only to HIV genotype I(for sub-genotype A~H).

Short operating time: 2 and a half hours totally

Good stability: kept for 12 months at –20℃; CV≤5%;

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Human immunodeficiency virus (HIV) is a retrovirus that can lead to acquired immunodeficiency syndrome (AIDS). Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. HIV infection in humans is now pandemic. As of January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimate that AIDS has killed more than 25 million people since it was first recognized on December 1, 1981, making it one of the most destructive pandemics in recorded history.

HIV-1 real time RT-PCR kit contains a specific ready-to-use system for HIV-1 detection (for sub-genotype A~H) through Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The master contains a Super Mix for the specific amplification of HIV-1 RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the HIV-1 RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction (PCR). Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified HIV-1 DNA fragment is performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. In addition, the kit can be used for identification of possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). Four quantitation standards are supplied, allows the determination of the gene load.

4. Kit Contents

	Ref.	Cap Color	Type of reagent	Presentation 25rxns
PCR System	1	Red	HIV-1 Super Mix	1 vial, 480µl
	2	Green	RT-PCR Enzyme Mix	1 vial, 28µl
	3	White	Molecular Grade Water	1 vial, 400µl
	4	Orange	Internal Control	1 vial, 30µl
QS ★ System	5	Pink	HIV-1 QS1 (5×10^7IU/ml)	1 vial, 20µl
	6	Purple	HIV-1 QS2 (5×10^6IU/ml)	1 vial, 20µl
	7	Orange	HIV-1 QS3 (5×10^5IU/ml)	1 vial, 20µl
	8	Yellow	HIV-1 QS4 (5×10^4IU/ml)	1 vial, 20µl

★QS：Quantitation Standard

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Real time PCR reaction tubes/plates
- Vortex mixer
- Pipets (0.5 µl – 1000 µl)
- Cryo-container
- Disposable gloves, powderless
- Sterile microtubes
- Biohazard waste container
- Refrigerator and freezer
- Sterile filter tips for micro pipets
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. ⚠Warnings and Precaution

Carefully read this instruction before starting the procedure.

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.

• Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
• Prepare quickly the Reaction mix on ice or in the cooling block.
• Set up two separate working areas: 1) isolation of the RNA/ DNA and 2) amplification/detection of amplification products.
• Pipets, vials and other working materials should not circulate among working units.
• Use always sterile pipette tips with filters.
• Wear separate coats and gloves in each area.
• Avoid aerosols

8. Sample Collection, Storage and transport

• Collected samples in sterile tubes;
• Specimens can be extracted immediately or frozen at -20°C to -80°C.
• Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 RNA-Extraction

Different brand RNA Extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

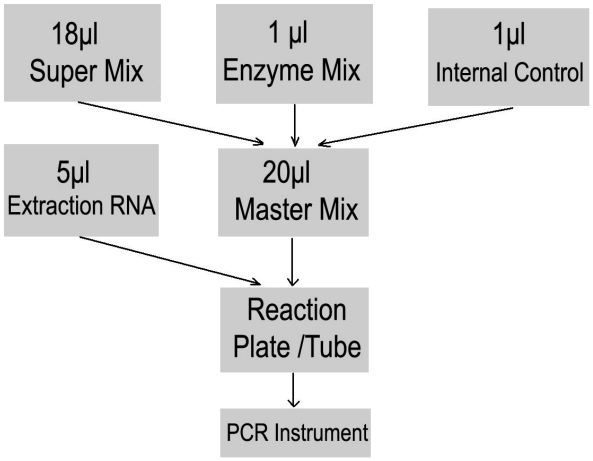
Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
RNA Isolation Kit	ME-0010/ME-0012	ZJ Biotech
QIAamp Viral RNA Mini extraction Kit (50)	52904	QIAGEN

9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal control (IC) allows the user to determine and control the possibility of PCR inhibition.

9.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



※ PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **20µl** Master Mix with micropipets of sterile filter tips to each of the *Real time* PCR reaction plate/tubes. Separately add **5µl** RNA sample supernatantor,QS1,QS2,QS3,QS4 and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

45°C for **10 min, 1 cycle; 95°C** for **15 min, 1 cycle; 95°C** for **15 sec, 60°C** for **60sec, 40 cycles.**
Fluorescence is measured at 60°C;FAM and HEX/VIC/JOE channels should be chosen.

5) ⚠If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

10. Threshold setting: just above the maximum level of molecular grade water.

11.Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12.Quality control:

Channel / Control	Ct value	
	FAM (Target Nucleic Acid)	HEX/VIC/JOE (IC)
Molecular Grade Water	UNDET	25~35
QS1/QS2/QS3/QS4	≤38，and Correlation coefficient of QS curve≤－0.98	——

13. Data Analysis and Interpretation

The following results are possible:

1) The Ct value in channel FAM shows ≤38. **The result is positive: The sample contains HIV-1 RNA. The sample contains HCV RNA. Quantitative value of samples is automatically reported according to the standard curve.**

Quantitative Value	Data Analysis and Suggestion
$< 1\times10^3$IU/ml	HIV-1 RNA Positive; its concentration lower than 10^3IU/ml
$10^3\sim2\times10^3$ IU/ml	HIV-1 RNA Positive; the quantitative value for recommendation only
$2\times10^3\sim10^8$ IU/ml	HIV-1 RNA Positive; the quantitative value is valid
$>10^8$ IU/ml	1) HIV-1 RNA Positive but the quantitative value for recommendation only 2)Re-test the sample after dilute the sample by several times, making the quantitative value within $2\times10^3\sim10^8$ IU/ml

2) The Ct value in channel FAM shows 38~40, please repeat again. **If the result still shows 38~40,it can be considered negative.**
3) In channel FAM no signal is detected, at the same time, a HEX/VIC/JOE signal from the Internal Control appears. **The sample does not contain any HIV-1 RNA. It can be considered negative.**
4) Neither in channel FAM nor in channel HEX/VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the RT-PCR reaction.

For further questions or problems，please contact our technical support at **trade@liferiver.com.cn**