PCR / RT-PCR Kit User's Manual

For HIV-1 RT- PCR Fluorescence Quantitative Detection

Preface

This kit combines the technologies of HIV-1 RNA extraction, RT-PCR, fluorescence quantitative detection and competitive internal control to quantify Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma, serum, blood products, etc.

Ingredients

Ingredients		BSB24S1	BSB24M1
Components		32 Tests	48 Tests
1	RT - PCR Reaction Solution	800μL	1200μL
2	Mn^{2+}	80μL	120μL
3	HIV-1 Primer /Probe	80μL	120μL
4	Positive control	500μL	500μL
5	Negative control	500μL	500μL
6	Internal control	250μL	250μL
7	Positive Reference 1：（1-5）$\times 10^6$Copies/mL	100μL	100μL
8	Positive Reference 2：（1-5）$\times 10^5$Copies/mL	100μL	100μL
9	Positive Reference 3：（1-5）$\times 10^4$Copies/mL	100μL	100μL
10	Positive Reference 4：（1-5）$\times 10^3$Copies/mL	100μL	100μL

Applied instrument

The kit can be applied to Bioer's Line-Gene series fluorescent quantitative PCR detection system and other manufacturers' similar fluorescent quantitative PCR detection systems. The instrument should contain at least two channels of FAM and HEX.

Storage and period of validity

1. PCR Reagent store at -20℃.
2. The kit can be stored for up to 12 months if all components are kept in the manner above.（please use the kit in the period of validity）.

Additional required reagents

1. Nuclease-free aerosol-preventive pipette tips.
2. Sterile centrifuge (Eppendorf) tube for preparing, 0.2 mL real-time PCR tube.

Sample collection, storage and transportation

1. Sample collection:
   Use vacuum blood collection tube and gyroidal needles or syringes to avoid the direct contact with blood.
   Collect blood from vein with disposable syringe or vacuum blood collection tube. Centrifuge the tube at 3000rpm for 15 minutes. Transfer the supernatant into sterile 1.5mL tube for sample extraction.
2. Sample storage:
   Store the sample at 2-8℃ for several days. For long time, store at -20℃. Store at -80℃ for prolonged up to 3 months.
3. Sample transportation:
   Transport the sample at 2~8℃.

Using of the kit

I. Sample extraction (sample disposal area)

1) You can use MagaBio plus Virus DNA/RNA Purification Kit (Cat#. BSC57/BSC71) to purify the HBV DNA with Gene Pure Series Nucleic acid extractor.
2) Add 200μL the serum and 5μL internal control to the pre-packed plates for the DNA extraction. The specific steps are detailed in the extraction operation manual.

II. PCR reaction (PCR test area)

1. Reagent prepares:
   Thaw out the reagents at room temperature. Before preparing RT-PCR reagents, mix gently and centrifuge all reagents for a few seconds.
   Make RT-PCR reagents according to the quantity of sample, controls and references as below (n tests add an extra blank control):

Reagents	RT-PCR Reaction Solution	Mn^{2+}	HIV-1 Primer /Probe
Dosage/ test	25 μL	2.5 μL	2.5 μL
Dosage	（n+1）× 25 μL	（n+1）× 2.5 μL	（n+1）× 2.5 μL

After mixing RT-PCR reagents above, distribute 30μL into every 0.2mL PCR tubes.

2. Adding sample
   Add 20μL extracted sample, controls or references into every PCR tube above.

3. RT-PCR reaction

Set reaction procedure as following:

90℃：30 sec；
61℃：20 min；
95℃： 1 min；
95℃：15 sec；
60℃：1 min；
} 45 cycles

Select the fluorescent channel of instrument for testing:
Choose F1（FAM）and F2 (HEX) channels to collect fluorescent signals.

4. Before running Line-Gene Series Real-time PCR detection system, set fluorescent signals detecting at 60℃ for 10 seconds, and then adjust gain to make the F1（FAM）and F2 (HEX) background between 3000-4000.

Result analysis and judgments

1. Line-Gene Series Real-time PCR detection system analysis: Select fit point method to analyze. Input the concentrations of four positive References. Confirm the base line 2 (zero adjustment) by getting the fluorescent signals of 10-15 cycles for F1（FAM）and 10-20 for F2 (HEX). Make the noise limit just beyond the peak of the amplification curve (rule less noise line) of normal negative control; then do quantitative analysis. You could also adjust by yourself according to the condition of instrument's noises.
2. If the result of 1×10^2copies/mL≤HIV-1 RNA≤1×10^7copies/mL, the result is available, and you can report relative copies directly.
3. If in the test sample, HIV-1 RNA ＞ 1×10^7copies/mL, you can report as ＞ 1×10^7copies/mL directly, or you can use HIV-1 negative serum to dilute as per 10 times of grads, then re-test when the copies is among 1×10^5~1×10^7copies/mL, adjusting test result by dilution times.
4. When the test sample's HIV-1 RNA＜1×10^2copies/mL, and the Ct value of F2 (HEX) channel ＜36, copies is just for your reference, while the report should be less than lowest detectable concentration. If the Ct value of F2 (HEX) channel ＞ 36, there may be some inhibitor in RT-PCR reaction and an re-test is recommended.
5. When the Ct value of F2 (HEX) channel ＜36 and the Ct value of F1（FAM） doesn't appear, the report is 0.0 copies /mL.

Quality Control

The absolute value of relative parameter for standard curve should be ≥0.970, Ct value of negative references shouldn't appear, the Ct value of positive reference ＜ 37 , the Ct value of Internal control ＜36; Otherwise, the experiment is unavailable.

Note

1. Please clarify the experiment areas according to the PCR demand, such as reagent preparation area, sample disposal area, and PCR test area. Don't confuse the instruments that belong to different areas.
2. Operators must take training before operation.
3. Don't touch hand directly when you use one-time consumables such as pipe and centrifuge tube.
4. Please use one-time PE gloves during the whole operation; don't open the tube after amplification, discard it into appointed containers.
5. Be caution during the whole process to ensure the accuracy of the experiment. After finishing the experiment, in order to avoid pollution, please use 75% alcohol to clean the worktable.
6. Samples should be stored at -20℃ and avoid freeze-thaw cycles.
7. Pay attention to the temperature and time of lysis. Inefficient lysis may affect the result of experiment.

Technical support:

For technical support, please dial phone number +86-571-87774567-5211 or 87774575, by fax to +86-571-87774553, or by email to reagent@bioer.com.cn.

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