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.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'-nuclease assay. During the PCR process, the DNA polymerase cleaves the reporter dye 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 (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time PCR 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 real-time assay 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 sequences by means of polymerase chain reaction (PCR).

4. Kit Contents

- 1 vial of Super Mix
- 1 vial of Super Mix
- 1 vial of Molecular Grade Water
- 2 µl of Internal Control (IC)
- A Positive Control (1×10^9 IU/ml) in a single tube

5. Analysis sensitivity: 1×10^9 IU/ml; LOQ: 2×10^7 – 1×10^10 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 kit recommended, the analysis sensitivity is the same as above. 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.

6. 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 (3×) 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.

7. Additional Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- RNA extraction kit
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile micropipets
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Tube racks

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:

<table>
<thead>
<tr>
<th>Nucleic Acid Isolation Kit</th>
<th>Cat. Number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIAamp Viral RNA Mini extraction kit (50)</td>
<td>52604</td>
<td>QIAGEN</td>
</tr>
</tbody>
</table>

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 Quantification

The kit can be used for quantitative or qualitative real-time RT-PCR. A positive control defined as ≥1×10^8 IU/ml is supplied in the kit.

To perform quantitative real-time PCR, standard dilutions must be prepared first as follows: Molecular Grade Water is used for dilution.

- The step of dilution is not needed for performance of qualitative real-time PCR.

For further information, please refer to section 9.3 Quantitation.

9.4 RT-PCR Protocol

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

1 µl Master Mix
1 µl Enzyme Mix
1 µl Internal Control
Ct value

To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

Attention: All kit should be pipetted thoroughly before next transfer.

8. The positive control (1×10^11 IU/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

8.4 PCR System

For in vitro diagnostic use only.

9.5 Quality control

- Negative control: Negative control, positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid.