### 1. Intended Use

HIV-2 real-time RT-PCR kit is used for the detection of HIV-2 in serum 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’ nucleic acid 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 fluorescence signal generated by the cleaved reporter dye. A quencher BHQ1. In addition, the kit can be used for identification of possible PCR inhibition by emitted and measured by the real time systems’ optical unit during the PCR. The detection of master contains Super Mix for the specific amplification of HIV.

### 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, breast milk, or mucosal or cutaneous lesion. In humans it is now pandemic. Two species of HIV infect humans: HIV-1 and HIV-2. HIV-1 is more virulent. It is easily transmittable and is largely confined to West Africa.

HIV-2 real-time RT-PCR kit contains a ready-to-use system for HIV-2 detection through Reverse Transcript Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The master contains Super Mix for the specific amplification of HIV-2 RNA including subtype A-G. The reaction is done in one step real-time RT-PCR. The first step is a reverse transcription (RT), during which the HIV-2 RNA is transcribed into cDNA. Then, a thermostable DNA polymerase is used to amplify the specific gene fragments by polymerase chain reaction (PCR). Fluorescence is emitted and measured by the real time systems’ optical unit during the PCR. The detection of amplified HIV-2 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). An external positive control defined as 1×10^5 IU/ml is supplied. It allows the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

### 4. Kit Contents

**Ref.** Type of reagent | Presentation | 25 rxns |
--- | --- | --- |
1 | HIV-2 Super Mix | 1 vial, 480 µl |
2 | RT-PCR Enzyme Mix | 1 vial, 20 µl |
3 | Molecular Grade Water | 1 vial, 40 µl |
4 | Internal Control (IC) | 1×10^3 IU/ml, 30 µl |
5 | HIV-2 Positive Control (1×10^1 IU/ml) | 1×10^3 IU/ml, 30 µl |

**Analysis sensitivity: 5×10^1 IU/ml:**

**LOQ: 1×10^3 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 buffer in the kit, 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.

### 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**
- **Vortex mixer**
- **Cryo-container**
- **Sterile filter tips for micro pipets**
- **Disposable gloves, powderless**
- **Biohazard waste container**
- **Tubing**
- **Desktop microcentrifuge for "appendend" 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 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.**

### 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>RNA Isolation Kit</td>
<td>ME-0010/ME-0012</td>
<td>ZJ BioTech</td>
</tr>
<tr>
<td>QIAamp Viral RNA Mini extraction Kit (50)</td>
<td>25904</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. Add the internal control (1) µl/ex and the result will be shown in the HEX/VIC/JOE.

#### 9.3 Quantitation

The kit can be used for quantitative or qualitative real-time RT-PCR. A positive control defined as ≥1×10^5 IU/ml is supplied in the kit.

#### 9.4 RT-PCR Protocol

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

<table>
<thead>
<tr>
<th>Dilution of Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 µl</td>
</tr>
</tbody>
</table>

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:

- **A.** Mix thoroughly before next transfer.
- **B.** The positive control (≥1×10^5 IU/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

### 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 assured:

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

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

#### 12.2 Positive control:

- Enter positive control (≥1×10^5 IU/ml) as the starting standard in the first tube. Respectively pipette 36 µl of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

![Diagram](attachment:image.png)

#### 12.3 Negative control:

- Enter negative control (≤1×10^4 IU/ml) as at least 20 cycles.

#### 12.4 Internal control:

- Enter internal control (≥1×10^3 IU/ml) as at least 20 cycles.

#### 12.5 Unidentified sample:

- Enter unidentified sample (≥UNDET) as at least 20 cycles.

#### 12.6 Re-test:

- If it is still 80–40, repeat as #14

#### 12.7 Below detection limit or negative:

- Enter below detection limit or negative sample (≤1×10^4 IU/ml) as at least 20 cycles.

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