Human Immunodeficiency Virus type 1
pol gene region
genesisig® Advanced Kit
150 tests

For general laboratory and research use only
Introduction to Human Immunodeficiency Virus type 1

Human immunodeficiency virus (commonly known as HIV, and formerly known as HTLV-III and lymphadenopathy-associated virus) is a retrovirus that is the cause of the disease known as AIDS (Acquired Immunodeficiency Syndrome), a syndrome where the immune system begins to fail, leading to many life-threatening opportunistic infections.

HIV is transmitted through direct contact of a mucous membrane with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, preseminal fluid or breast milk. This transmission can come in the form of: penetrative (anal or vaginal) sex; oral sex; blood transfusion; contaminated needles; exchange between mother and infant during pregnancy, childbirth, or breastfeeding; or other exposure to one of the above bodily fluids.

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. In 2005 alone, AIDS claimed an estimated 2.4—3.3 million lives, of which more than 570,000 were children. [1] A third of these deaths are occurring in sub-Saharan Africa, retarding economic growth by destroying human capital. Current estimates state that HIV is set to infect 90 million people in Africa, resulting in a minimum estimate of 18 million orphans.[2] Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection, but routine access to antiretroviral medication is not available in all countries.[3]

Two species of HIV infect humans: HIV-1 and HIV-2. HIV-1 is hypothesized to have originated in southern Cameroon after jumping from wild chimpanzees (Pan troglodytes troglodytes) to humans during the twentieth century. HIV-2 is hypothesized to have originated from the Sooty Mangabey (Cercocebus atys), an Old World monkey of Guinea-Bissau, Gabon, and Cameroon. HIV-1 is more virulent, more easily transmitted and is the cause of the majority of HIV infections globally, while HIV-2 is less easily transmitted and is largely confined to West Africa.

References
Specificity

The Primerdesign genesig Kit for Human Immunodeficiency Virus type 1 (HIV1) genomes is designed for the in vitro quantification of HIV1 genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

HIV-1 primers have been designed for the specific and exclusive in vitro quantification of all HIV-1 group M subtypes and does not detect groups N and O. The target sequence (pol) is highly conserved and has previously been shown to be a good genetic marker for HIV-1 detection in other real time PCR based studies (Tang N et.al 2007). The primers and probe sequences in this kit have 100% homology with over 95% of reference sequences contained in the NCBI database based on a comprehensive bioinformatics analysis. Representative sequences from each subtype are included in the polygenetic tree below.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Fig.1  Accession numbers for detected HIV-1 isolates
Kit contents

- HIV1 specific primer/probe mix (150 reactions BROWN)  
  FAM labelled

- HIV1 positive control template (for Standard curve RED)

- Internal extraction control primer/probe mix (150 reactions BROWN)  
  VIC labelled as standard

- Internal extraction control RNA (150 reactions BLUE)

- Endogenous control primer/probe mix (150 reactions BROWN)  
  FAM labelled

- RNase/DNase free water (WHITE)  
  for resuspension of primer/probe mixes

- Template preparation buffer (YELLOW)  
  for resuspension of internal control template, positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit
This kit is recommended for use with genesig Easy DNA/RNA Extraction kit. However, it is  
designed to work well with all processes that yield high quality RNA and DNA with minimal  
PCR inhibitors.

\textbf{oasig}^{TM} \textit{lyophilised OneStep or Precision}^{\textregistered}\textit{PLUS OneStep 2X RT-qPCR Master Mix}  
Contains complete OneStep RT-qPCR master mix

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes
Kit storage and stability
This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.
If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.
Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material
All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test
Under optimal PCR conditions genesig HIV1 detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers
This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer’s recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR.
Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5’ nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks
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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. Bi, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applera Genomics (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the Primerdesign™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.
Principles of the test

Real-time PCR
A HIV1 specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the HIV1 cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5'-dye and a 3'-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control
For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of HIV1 copy number / Cq value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target HIV1 gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control
To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.
Internal RNA extraction control
When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

A separate qPCR primer/probe mix are supplied with this kit to detect the exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the HIV1 target cDNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/−3 depending on the level of sample dilution.

Endogenous control
To confirm extraction of a valid biological template, a primer and probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel and it is NOT therefore possible to perform a multiplex with the HIV1 primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.
Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Resuspend the primer/probe mixes in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

<table>
<thead>
<tr>
<th>Component - resuspend in water</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-PCR pack</td>
<td></td>
</tr>
<tr>
<td>HIV1 primer/probe mix (BROWN)</td>
<td>165 µl</td>
</tr>
<tr>
<td>Internal extraction control primer/probe mix (BROWN)</td>
<td>165 µl</td>
</tr>
<tr>
<td>Endogenous control primer/probe mix (BROWN)</td>
<td>165 µl</td>
</tr>
</tbody>
</table>

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

<table>
<thead>
<tr>
<th>Component - resuspend in template preparation buffer</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-PCR heat-sealed foil</td>
<td></td>
</tr>
<tr>
<td>Internal extraction control RNA (BLUE)</td>
<td>600 µl</td>
</tr>
<tr>
<td>Post-PCR heat-sealed foil</td>
<td></td>
</tr>
<tr>
<td>HIV1 Positive Control Template (RED) *</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

**DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.**

1. Add 4µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.

2. Complete RNA extraction according to the manufacturer’s protocols.
OneStep RT-qPCR detection protocol

For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. For each RNA sample prepare a reaction mix according to the table below:
Include sufficient reactions for positive and negative controls.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix</td>
<td>10 µl</td>
</tr>
<tr>
<td>HIV1 primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Internal extraction control primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNase/DNase free water (WHITE)</td>
<td>3 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td>15 µl</td>
</tr>
</tbody>
</table>

2. For each RNA sample prepare an endogenous control reaction according to the table below (optional):
This control reaction will provide crucial information regarding the quality of the biological sample.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>oasig OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix</td>
<td>10 µl</td>
</tr>
<tr>
<td>Endogenous control primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNase/DNase free water (WHITE)</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td>15 µl</td>
</tr>
</tbody>
</table>

3. Pipette 15µl of these mixes into each well according to your qPCR experimental plate set up.

4. Pipette 5µl of RNA template into each well, according to your experimental plate set up.
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.
5. If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>oasisg OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix</td>
<td>10 µl</td>
</tr>
<tr>
<td>HIV1 primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNase/DNase free water (WHITE)</td>
<td>4 µl</td>
</tr>
</tbody>
</table>

| Final Volume                                          | 15 µl  |

6. Preparation of standard curve dilution series.

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10 µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

7. Pipette 5µl of standard template into each well for the standard curve according to your plate set-up

The final volume in each well is 20µl.

**OneStep RT-qPCR Amplification Protocol**

Amplification conditions using oasisg OneStep or PrecisionPLUS OneStep 2X RT-qPCR Master Mix.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>10 min</td>
<td>55 °C</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>2 min</td>
<td>95 °C</td>
</tr>
<tr>
<td>Cycling x50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>10 s</td>
<td>95 °C</td>
</tr>
<tr>
<td><strong>DATA COLLECTION</strong></td>
<td>60 s</td>
<td>60 °C</td>
</tr>
</tbody>
</table>

* Fluorogenic data should be collected during this step through the FAM and VIC channels
## Interpretation of results

<table>
<thead>
<tr>
<th>Target (FAM)</th>
<th>Internal control (VIC)</th>
<th>Positive control</th>
<th>Negative control</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30</td>
<td>+ / -</td>
<td>+</td>
<td>-</td>
<td>POSITIVE QUANTITATIVE RESULT calculate copy number</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>POSITIVE QUANTITATIVE RESULT calculate copy number</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>POSITIVE QUALITATIVE RESULT do not report copy number as this may be due to poor sample extraction</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NEGATIVE RESULT</td>
</tr>
</tbody>
</table>

| + / - | + / - | + | ≤ 35 | EXPERIMENT FAILED due to test contamination |
| + / - | + / - | + | > 35 | * |
| -    | -    | + | -    | SAMPLE PREPARATION FAILED |
| + / - | + / - | - | + / - | EXPERIMENT FAILED |

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:

**SAMPLE POSITIVE**

If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.

**INCONCLUSIVE**

If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.
Internal PCR control
The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a HIV1 sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Endogenous control
The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.