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HIV Proviral DNA PCR Kit — Product Insert

Product # 33840

Background Information

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Infection with HIV occurs by the transfer of bodily fluids including blood, semen, vaginal fluid, pre-ejaculate, or breast milk. The lifecycle of HIV consists of the injection of its RNA into human cells and conversion into complementary DNA (cDNA) via reverse transcription. The integration of cDNA strands into the host cell genome is known as the proviral form of HIV. The mRNA that is transcribed from the proviral DNA is used to create new viral proteins that are used to build new viruses, thereby completing the replication process. Within hours of infection, proviral DNA is present. This initial stage of HIV infection prior to antibody response is known as an acute HIV infection. Testing for proviral DNA is used to determine if an individual who has had high risk exposure with someone known to have HIV has contracted it themselves, thus shortening the "window period" for testing. Moreover, HIV proviral DNA test could help resolve inconclusive blood test such as in the case of low viral load in patients undergoing treatment or suppressive therapy. Finally, HIV proviral DNA detection is very useful for HIV diagnosis in infants under 2 years of age born to infected mothers.

Product Description

Norgen's HIV Proviral DNA PCR Kit is a research use-only test, based on the use of end-point PCR technology, for the detection of HIV proviral DNA. The kit includes Master Mix and primers for the specific amplification of a 142 bp region of the HIV proviral DNA. In addition, the kit contains a positive and a negative control to confirm the integrity of the kit reagents.

The detection of HIV proviral DNA is based on end-point PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of specific HIV proviral DNA sequences. For analysis of the PCR data, the PCR reaction is loaded on an agarose DNA gel along with the provided DNA marker for qualitative analysis.

Norgen's HIV proviral DNA PCR Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad iCycler

Kit Components

Component	Product # 33840 (48 preps)
2X PCR Master Mix	1 mL
HIV Proviral DNA Primer Set Mix	300 µL
HIV Proviral DNA Positive Control	100 µL
Nuclease-Free Water	1.25 mL
DNA Ladder	200 µL
Product Insert	1

Storage Conditions and Product Stability

- The HIV Proviral DNA PCR Kit is shipped on dry ice. The components of the kit should be frozen upon arrival. If one or more of the components is not frozen when the kit is received, or if any of the components have been compromised during shipment, please contact Norgen Biotek for assistance.
- All kit components should be stored at -20°C upon arrival

- Repeated thawing and freezing (> 2 x) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
- These reagents should remain stable for at least 1 year when stored at the specified conditions.

Customer-Supplied Reagents and Equipment

- Appropriate End-point PCR Instrument
- DNA Purification Kit
    - The kit is compatible with all DNA purification kits that yield high quality, inhibitor-free DNA
    - **Recommended Purification Kit:** Norgen Biotek's purification kits for DNA isolation, including:
        - Blood Genomic DNA Isolation Mini Kit - Cat# 46300
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- Agarose gel electrophoresis apparatus
- UV transilluminator with suitable gel documentation system

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's HIV Proviral DNA PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Do not substitute or mix reagents from different kit lots or from other manufacturers. Do not use components of the kit that have passed their expiration date.
- The presence of PCR inhibitors may cause false negative or invalid results.

- Potential mutations within the target regions of the HIV Proviral DNA genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
- Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

Instructions for Use

A. Sample Preparation

Purified DNA is the starting material for Norgen's HIV Proviral DNA PCR Kit. The quality of the DNA template will have a major impact on the performance of the diagnostic test. The user must ensure that the method used for DNA purification is compatible with end-point PCR technology. We recommend the use of Norgen's purification kits for DNA isolation, including **Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300)**.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 10 minutes at 14,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the DNA. This will help to prevent the carry-over of any ethanol into the purified DNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the DNA.**

B. PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The amount of 2X PCR Master Mix provided is enough for up to 64 PCR reactions (48 sample PCR, 8 positive control PCR and 8 no template control PCR).
- For every PCR run, one reaction containing HIV Proviral DNA Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume of sample DNA than recommended may affect the sensitivity of the HIV Proviral DNA Limit of Detection.
- To avoid any contamination while preparing the PCR assay, follow the order outlined in Tables 1, 2 and 3 below to prepare the Negative Control, Detection Assay and Positive Control:
    1. Prepare the Negative Control PCR (Table 1)
    2. Prepare the HIV Proviral DNA Assay PCR (Table 2)
    3. Prepare the Positive Control PCR (Table 3)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) Master Mix; 3) Primer Set; and 4) the Sample DNA or Positive Control).

1. For each PCR set, prepare **one** no template control PCR as shown in Table 1 below:

Table 1. Negative Control PCR Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	8 µL
2X PCR Master Mix	10 µL
HIV Proviral DNA Primer Set Mix	2 µL
Total Volume	20 µL

2. Prepare the PCR reaction for sample detection as shown in Table 2 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 2. HIV Proviral DNA Assay PCR Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	5.5 µL
2X PCR Master Mix	10 µL
HIV Proviral DNA Primer Set Mix	2 µL
Sample DNA	2.5 µL
Total Volume	20 µL

3. For each PCR set, prepare **one** positive control PCR as shown in Table 3 below:

Table 3. Positive Control PCR Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	3 µL
2X PCR Master Mix	10 µL
HIV Proviral DNA Primer Set Mix	2 µL
HIV Proviral DNA Positive Control (PosC)	5 µL
Total Volume	20 µL

C. HIV Proviral DNA PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

Table 4. HIV Proviral DNA PCR Assay Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	95°C	3 min
Cycle 2 (40x)	Step 1	94°C	15 sec
	Step 2	60°C	30 sec
	Step 3	72°C	45 sec
Cycle 3	Step 1	72°C	5 min
Cycle 4	Step 1	4°C	∞

D. HIV Proviral DNA PCR Assay Interpretation

- For the analysis of the PCR data, the entire 20 µL PCR reaction should be loaded on a 1X TAE 2% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
- The PCR products should be resolved on the 1X TAE, 2% Agarose gel at 150V for 30 minutes (Gel running time will vary depending on an electrophoresis apparatus).

Valid Test Run

- **Positive Sample**: A sample is determined to be positive only when:
  - Sample lanes shows the 142 bp band corresponding to the HIV Proviral DNA target amplicon
  - Positive Control shows the 142 bp band
  - Negative Control shows no bands
- **Negative Sample**: A sample is determined to be negative only when:
  - Sample lanes contain no bands
  - Positive Control shows the 142 bp band
  - Negative Control shows no bands

Invalid Test Run

- A test run is invalid if:
  - The run has not been completed
  - Positive Control does not show the 142 bp band
  - Negative Control shows any amplification

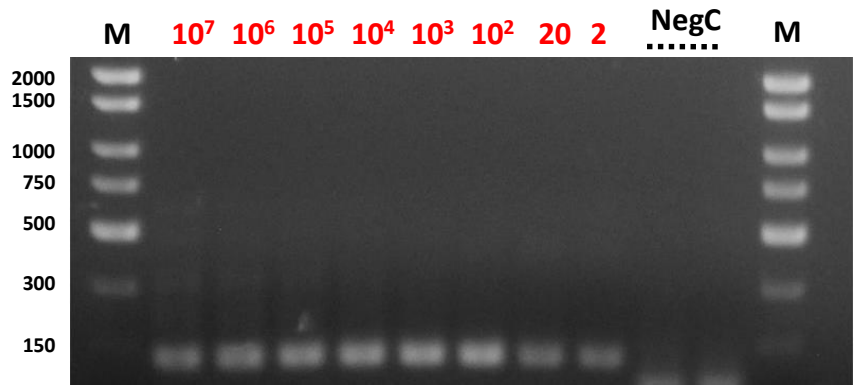

Figure 1: A representative 1X TAE 2% agarose gel showing the amplification of HIV. The size of the HIV target amplicon corresponds to the 142 bp band represented by the provided DNA Marker (M). No amplification of the target is observed in with the Negative Control

E. Specificity

The specificity of Norgen's HIV Proviral DNA PCR Kit is first and foremost ensured by the selection of the HIV Proviral DNA-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis. The specific detectability of all relevant strains has thus been ensured by a database alignment and by PCR amplification with the following commonly-found pathogens: *Pneumocystis jirovecii Neisseria gonorrhoea*, *Chlamydia trachomatis*, Norovirus, West Nile Virus.

F. Linear Range

- The linear range (analytical measurement) of Norgen's HIV Proviral DNA PCR Kit was determined by analysing a dilution series of an HIV proviral DNA quantification standard ranging from 1 x 10^7 copies/µl to 1 x 10^{-1} copies/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's HIV Proviral DNA PCR Kit on 1X TAE 1.7% Agarose gel.
- The linear range of Norgen's HIV Proviral DNA PCR Kit PCR Kit has been determined to cover concentrations from 1 x 10^2 copies/µl to at least 1 x 10^6 copies/µl of isolated DNA

G. Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

Norgen's HIV Proviral DNA PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR and in vitro diagnostic procedures.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

The presence of PCR inhibitors may cause false negative or invalid results.

Potential mutations within the target regions of the HIV Proviral DNA genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

The respective user is liable for any and all damages resulting from application of Norgen's HIV Proviral DNA PCR Kit for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

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