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HIV Quantitative RT-PCR Detection Kit — Product Insert

Product # 33740

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 blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (vertical transmission). Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world. HIV infection in humans is considered pandemic by the World Health Organization (WHO). Since its discovery in 1981, AIDS has infected over 78 million people, and 39 million people have died. Currently, an estimated 0.8% of adults aged 15–49 years worldwide are living with HIV. In 2013 alone, AIDS claimed an estimated 1.5 million lives, and an estimated 2.1 million people were newly infected with HIV. HIV infects primarily vital cells in the human immune system such as helper T cells (CD4+ T cells), macrophages, and dendritic cells. HIV progresses to AIDS at a variable rate affected by viral, host, and environmental factors; HIV-specific treatment delays this process. Most people will progress to AIDS within 10 years of HIV infection; however others will progress much sooner while some will take much longer. Treatment with anti-retrovirals increases the life expectancy of people infected with HIV. In 2013, around 12.9 million people living with HIV (37% of the total) had access to antiretroviral therapy.

Product Description

Norgen's HIV Quantitative RT-PCR Kit is a research use-only diagnostic test for the detection of HIV-specific RNA transcripts. The kit could be used for quantification of HIV RNA using end-point RT-PCR (gel electrophoresis-based) or real-time RT-PCR (detected via SYBR Green I) with the Primer Set and the Master Mix provided with the kit via amplification of a 142 nt region of the HIV RNA genome. In addition, the kit contains a quantified Positive Control (PosC, 20,000 copies per µL) that can be used for construction of a dilution series for HIV RNA quantification.

Norgen's HIV Quantitative RT-PCR Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad iCycler
- BioRad CFX96 Touch

Kit Components

Component	Product # 33740 (48 Samples)
2X Real-Time RT-PCR Master Mix	1 mL
HIV Primer Set Mix	300 µL
HIV Positive Control	100 µL
Nuclease-Free Water	1.25 mL
DNA Ladder	200 µL
Product Insert	1

Storage Conditions and Product Stability

- The HIV Quantitative RT-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 or Real-Time PCR Instrument
- RNA Purification Kit
    - The kit is compatible with all RNA purification kits that yield high quality, inhibitor-free DNA
    - **Recommended Purification Kit:** Norgen Biotek's purification kits for RNA isolation, including:
        - Total RNA Purification Kit - Cat# 17200
        - Plasma /Serum Circulating RNA and Exosomal Purification Kit - Cat# 42800
        - Plasma/Serum RNA Purification Kit - Cat# 55000
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- Agarose gel electrophoresis apparatus (End-Point PCR)
- UV transilluminator with suitable gel documentation system (End-Point PCR)

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's HIV Quantitative RT-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 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 RNA is the starting material for Norgen's HIV Quantitative RT-PCR Kit. The quality of the RNA template will have a major impact on the performance of the diagnostic test. The user must ensure that the method used for RNA purification is compatible with RT-PCR technology. We recommend the use of Norgen's purification kits for RNA isolation, including **Norgen's Total RNA Purification Kit (Cat# 17200), Plasma/Serum Circulating RNA and Exosomal Purification Kit (Cat# 42800)**, **Plasma/Serum RNA Purification Kit (Cat# 55000).**

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 RNA. This will help to prevent the carry-over of any ethanol into the purified RNA, 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 RNA.**

B. RT-PCR Assay Preparation

Notes Before Use:

- Before use, suitable amounts of all RT-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 Real-Time RT-PCR Master Mix provided is enough for up to 96 RT-PCR reactions (48 sample RT-PCR, 32 positive control/standard curve RT-PCR and 16 no template control RT-PCR).
- For every RT-PCR run, one reaction containing the non-diluted HIV Positive Control and one reaction as no template control must be included for proper interpretation of results.
- For quantitative interpretation, a dilution series of the positive control RNA should be generated (instruction provided below).
- The recommended minimum number of RNA samples tested per RT-PCR run is 6.

- Using a lower volume of sample RNA than recommended may affect the sensitivity of the HIV Limit of Detection.
- To avoid any contamination while preparing the RT-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 RT-PCR Negative Control (Table 1)
    2. Prepare the RT-PCR HIV Assay (Table 2)
    3. Prepare the RT-PCR Positive Control Dilution Series (Table 3)
    4. Prepare the RT-PCR Positive Control Assay (Table 4)
- 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 RNA or Positive Control).

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

Table 1. RT-PCR Negative Control Preparation

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

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

Table 2. RT-PCR *HIV* Assay Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
Nuclease-Free Water	5.5 µL
2X Real-Time RT-PCR Master Mix	10 µL
HIV Primer Set Mix	2 µL
Sample RNA	2.5 µL
Total Volume	20 µL

3. For each RT-PCR set, prepare a Positive Control dilution series as shown in Table 3 below:

Table 3. RT-PCR Positive Control Dilution Series Preparation

HIV copies per µL	Volume of Nuclease-Free Water	Volume of PosC of Different Concentration
2×10^4	Original **PosC**. No dilution Required	Original **PosC**. No dilution Required
2×10^3	18 µL	2 µL of **PosC** (2×10^4)
2×10^2	18 µL	2 µL of **PosC** (2×10^3)
2×10^1	18 µL	2 µL of **PosC** (2×10^2)
2×10^0	18 µL	2 µL of **PosC** (2×10^1)

4. Using the Positive Control dilution series prepared above, prepare positive control PCRs as shown in Table 4 below:

Table 4. RT-PCR Positive Controls Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	3 µL
2X Real-Time RT-PCR Master Mix	10 µL
HIV Primer Set Mix	2 µL
HIV Positive Control (PosC) or Dilution Series	5 µL
Total Volume	20 µL

NOTE: Set up one reaction for each of the PosC dilution

C. HIV RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 5 below.
2. Run one step RT-PCR.

Table 5. HIV Assay Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**50°C**	**25 min**
Cycle 2	**Step 1**	**95°C**	**5 min**
Cycle 3 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 4	**Step 1**	**72°C**	**5 min**
Cycle 5	**Step 1**	**4°C**	**∞**

D. HIV RT-PCR Assay Interpretation

- For real-time analysis, use the analysis software of the thermocycler to generate a standard curve using the Ct values of the Positive Control Dilution Series. The standard curve can then be used to determine the starting quantity of the sample of interest.
- For the analysis of the end-point RT-PCR data, the entire 20 µL RT-PCR reaction should be loaded on a 1X TAE 2% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
- The RT-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 target amplicon
  - Positive Control shows the 142 bp band
  - Positive Control shows the 142 bp band even it is diluted to as little as 20 copies per µL
  - 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

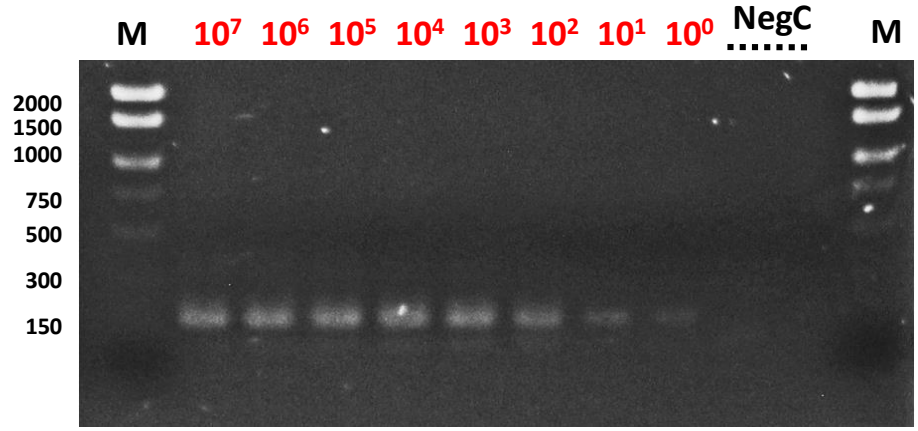

Figure 1: A representative 1X TAE 2% agarose gel showing the amplification of HIV. The size of the HIV target amplicon corresponds to the 142 np bp band represented by the provided DNA Marker (M). No amplification of the target is observed in with the Negative Control

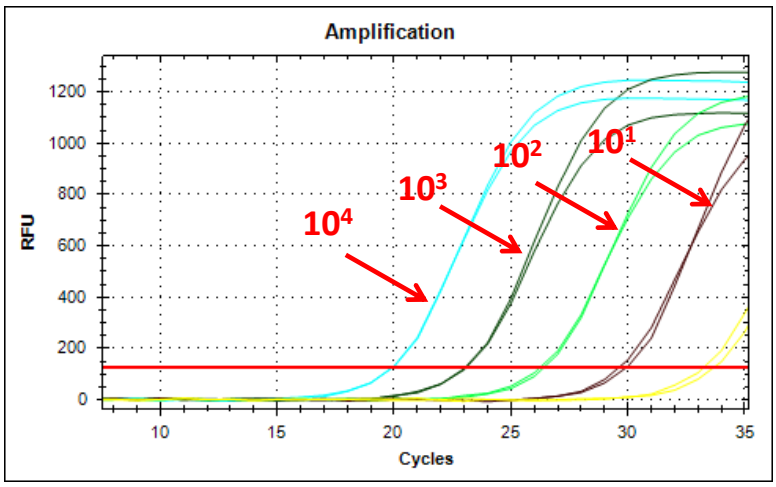

Figure 2: A representative RT-qPCR baseline graph showing the successful amplification of a dilution series of HIV Positive Control.

E. Specificity

The specificity of Norgen's HIV Quantitative RT-PCR Kit is first and foremost ensured by the selection of the HIV-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, HIV.

F. Linear Range

- The linear range (analytical measurement) of Norgen's HIV Quantitative RT-PCR Kit was determined by analysing a dilution series of a HIV quantification standard ranging from 1 x 10^7 copes/µl to 1 x 10^{-1} copies/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's HIV Quantitative RT-PCR Kit on 1X TAE 1.7% Agarose gel.
- The linear range of Norgen's HIV Quantitative RT-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

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 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 Quantitative RT-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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