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HIV Quantitative TaqMan RT-PCR Detection Kit Product Insert

Product # TM33740

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 a 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 TaqMan RT-PCR Detection Kit is a research use only kit designed for the detection of HIV-specific RNA transcripts. The kit contains One-Step RT-PCR Master Mix and HIV specific Primer & Probe Mix (FAM fluorophore) for detection via amplification of a 123 nt region of the HIV RNA genome. In addition, the kit contains a quantified Positive Control (250,000 copies/µL) that can be used for construction of a dilution series for HIV RNA quantification.

This kit is designed for research use only and not for use in diagnostic procedures.

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

- Qiagen Rotor-Gene Q
- BioRad iCycler
- BioRad CFX96 Touch
- Applied Biosystems QuantStudio™ 7Pro

Kit Components

Component	Product # TM33740 (48 Samples)
2X One-Step RT-PCR Master Mix	3 x 350 µL
HIV Primer & Probe Mix	3 x 70 µL
HIV Quantified RNA Standard	100 µL
Nuclease-Free Water	1.25 mL
Product Insert	1

Storage Conditions and Product Stability

- The HIV Quantitative TaqMan RT-PCR Detection Kit is shipped on dry ice.
- All kit components should be stored at -20°C 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.
- 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 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
- PCR reaction preparation station (Optional)

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's HIV Quantitative TaqMan RT-PCR Detection 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 TaqMan RT-PCR Detection 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 One-Step RT-PCR Master Mix provided is enough for up to 96 RT-PCR reactions (48 samples, 6 positive control standard curves, and 12 no template controls).
- 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 8.
- 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 One-Step RT-PCR Master Mix	10 µL
HIV Primer & Probe 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 One-Step RT-PCR Master Mix	10 µL
HIV Primer & Probe Mix	2 µL
Sample RNA	2.5 µL*
Total Volume	20 µL

* Note: A sample volume of 8 µL from high quality RNA can be used for detection.

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

Volume of Nuclease-Free Water	Volume of PosC of Different Concentration	HIV copies/µL	HIV copies in 8 µL (used PosC volume/reaction)
Original **PosC**. No dilution Required	Original **PosC**. No dilution Required	250,000	2×10^6
18 µL	2 µL of **PosC** 250,000 copies/µL	25,000	2×10^5
18 µL	2 µL of **PosC** 25,000 copies/µL	2,500	2×10^4
18 µL	2 µL of **PosC** 2,500 copies/µL	250	2×10^3
18 µL	2 µL of **PosC** 250 copies/µL	25	2×10^2
18 µL	2 µL of **PosC** 25 copies/µL	2.5	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
2X One-Step RT-PCR Master Mix	10 µL
HIV Primer & Probe Mix	2 µL
HIV Quantified RNA Std. or Dilution Series	8 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**	**30 min**
Cycle 2	**Step 1**	**95°C**	**3 min**
Cycle 3 (40x)	**Step 1**	**95°C**	**15 sec**
	Step 2	**60°C**	**30 sec**

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.

Valid Test Run

- **Positive Sample**: A sample is determined to be positive only when:
  - All (6) positive control dilutions are amplified in the RT-PCR reaction.
  - Negative control shows no amplification.
  - Sample wells contain a Ct less than the lowest diluted standard at 20 copies in the final 20 µL reaction (8µL x 2.5 copies/µL = 20 copies)
- **Negative Sample**: A sample is determined to be negative only when:
  - All (6) positive control dilutions are amplified in the RT-PCR reaction.
  - Negative control shows no amplification.
  - Sample wells show no amplification.

Invalid Test Run

- A test run is invalid if:
  - If one or more of the (6) positive control dilutions are not amplified in the RT-PCR reaction.
  - Negative control shows any amplification.

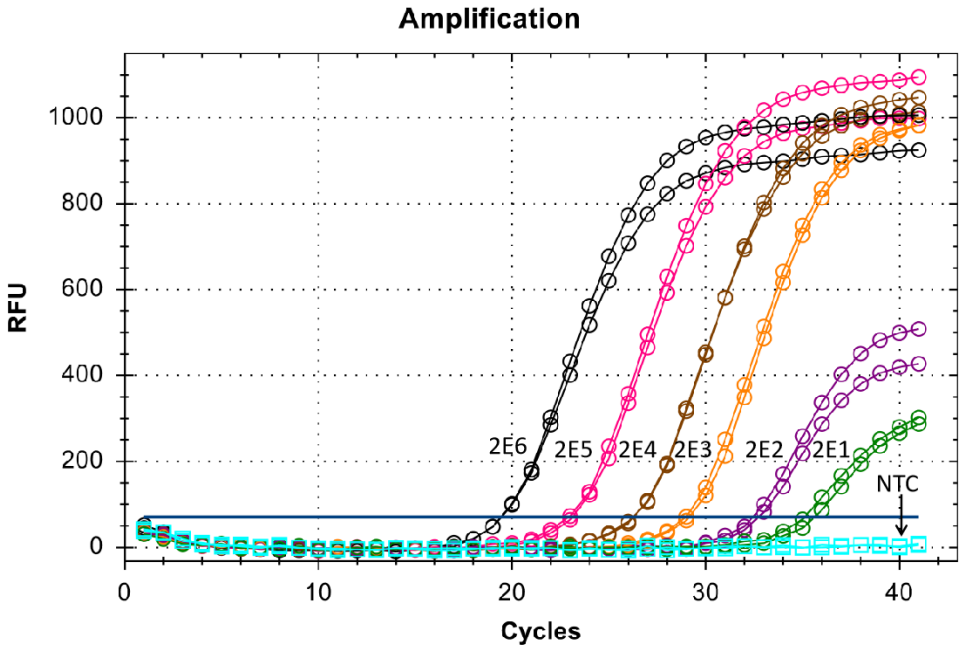

Figure 1: A representative RT-qPCR baseline graph showing the successful amplification of a dilution series of HIV Positive Control.

E. Specificity

The specificity of Norgen's HIV Quantitative TaqMan RT-PCR Detection Kit is first and foremost ensured by the selection of the HIV-specific primer & probe mix, as well as the selection of stringent reaction conditions. The primers and probe 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 TaqMan RT-PCR Detection Kit was determined by analysing a dilution series of a HIV quantification standard ranging from 2 x 10^6 copies to 2 x 10^1 copies.
- Each dilution has been tested in replicates (n = 4) using Norgen's HIV Quantitative TaqMan RT-PCR Detection Kit.

G. Technical Support

Contact our Technical Support Team between the hours of 9:00am and 5:30pm (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

This kit is designed for research use only and not for use in 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 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 TaqMan RT-PCR Detection 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.

TaqMan is a registered trademark of Roche Molecular Systems, Inc

QuantStudio is a registered trademark of Life Technologies, Corp.

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