Rev.0

HIV-1 Virus One-step RT-PCR Detection Kit (DHIV1)

Catalog #RU7168
100 reactions

Product Description

HIV-1, human immunodeficiency virus 1, is a type of retrovirus that attacks the human immune system. If HIV-1 infection is not treated with anti-retroviral drugs, it can cause Acquired Immunodeficiency Syndrome, otherwise known as AIDS. There are four groups of HIV-1 virus: M, N, O, and P. The group M is further divided into nine subtypes: A, B, C, D, F, G, H, J, and K. ScienCell's HIV-1 Virus One-step RT-PCR Detection Kit (DHIV1) is designed to detect the presence of HIV-1 virus in cultured cells by one-step reverse transcription PCR (RT-PCR), followed by the electrophoresis analysis of the PCR product. The HIV-1 primer set (included in Cat #7168-ps) targets all four groups, including nine group M subtypes of HIV-1, and gives a positive band at 175 bp. The human control primer set (also included in Cat #7168-ps) targets human B2M gene RNA, but not genomic DNA, with a positive band of 85 bp and acts as a control to assess specimen quality. The positive control (Cat # RU011-pos) consists of non-infectious HIV-1 RNA fragment spiked into human primary cells and serves to ensure reagents and instruments are working properly. In addition, RubyNStart one-step RT-PCR master mix (Cat #MB6058a), RubyNStart enhancer (Cat #MB6058b), cell lysis buffer (Cat #GQ400a), cell lysis buffer enhancer (Cat #GQ400b), and nuclease-free water (Cat #GQ100-4) are included in the kit. Please refer to Tables 4 and 5 for results interpretation.

Kit Components

Cat #	Component	Quantity	Storage
GQ400a	Cell Lysis Buffer	25 mL	4°C
GQ400b	Cell Lysis Buffer Enhancer, 100x	250 µL	-20°C
7168-ps	HIV-1 and Human Control Primer Sets	200 µL	-20°C
MB6058a	RubyNStart One-Step RT-PCR Master Mix, 4X	500 µL	-20°C
MB6058b	RubyNStart Enhancer, 2X	1 mL	-20°C
RU011-pos	Positive Control (non-infectious; RNA: 5,000 – 10,000 copies/µL, cells: 10,000 counts/µL)	40 µL	-80°C
GQ100-4	Nuclease-Free H_2O	4 mL	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
RNA extraction kit (optional)	Viral RNA Isolation Kit (ScienCell, Cat #MB891)
PCR plate or tube	

Quality Control

The primer sets and the positive control are validated by one-step RT-PCR using serially diluted templates. The PCR products are analyzed by 1.5% agarose gel electrophoresis.

Product Use
For Research Use Only. Not for use in diagnostic procedures.

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store the positive control (Cat #RU011-pos) at -80°C; store the RubyNStart one-step RT-PCR master mix and its enhancer (Cat #MB6058a and MB6058b), cell lysis buffer enhancer (Cat #GQ400b), and HIV-1 and human control primer sets (Cat #7168-ps) at -20°C in a manual defrost freezer; and store cell lysis buffer (Cat #GQ400a) and nuclease-free H_2O (Cat #GQ100-4) at 4°C. Aliquot as needed to avoid repeated freeze-and-thaw cycles and cross-contamination among trials.

Procedures

Important: *Only use nuclease-free reagents in PCR applications.*

A. Preparation of cell lysate samples

Note: Skip Section A if using purified RNA as the RT-PCR template. When purifying RNA from positive control (Cat # RU011-pos) using an RNA extraction kit, take 2 uL of the positive control (Cat # RU011-pos) as the staring material, and elute with 30 µL of H_2O in the final elution step.

1. For each sample, count the number of cells to be harvested. Harvesting 0.1-2 million cells per sample is recommended. Wash cells with PBS once, pellet cells, and carefully remove PBS.

2. Determine the total volume of cell lysis buffer (Cat #GQ400a) to be used for the test samples at 1,000-5,000 cells/µL cell lysis buffer (see an "example of calculations" below). Transfer the calculated amount of cell lysis buffer with 10% extra to a new tube. Supplement the aliquoted cell lysis buffer with cell lysis buffer enhancer (100x, Cat #GQ400b). For every milliliter of cell lysis buffer, add 10 µL of cell lysis buffer enhancer.
   Note: One positive control sample should be prepared with each RT-PCR run. To prepare the positive control sample, take 2 uL of the positive control (Cat # RU011-pos) and lyse with 18 µL of supplemented cell lysis buffer.

3. Transfer the supplemented cell lysis buffer to each cell pellet sample at 1,000-5,000 cells/µL supplemented cell lysis buffer. Carefully pipette the cell pellet up and down 20 times without generating bubbles. The samples should be homogenous. If not, continue pipetting until fully homogenized. For the positive control sample, transfer 18 µL of supplemented cell lysis buffer to 2 uL of the positive control (Cat # RU011-pos), and carefully pipette up and down 20 times without generating bubbles.

4. Incubate the homogenized samples at 55°C for 30 minutes, followed by incubating at 95°C for 10 minutes to fully lyse the samples. Alternatively, transfer 20 µL of each homogenized sample from step A.3 to a PCR tube, and run a PCR program as shown in Table 1. Keep lysed samples on ice for immediate testing or store at -80°C. Storage time over 6 months is not recommended.

Table 1. PCR program settings for lysing the cells.

Step	Temperature	Time	Number of cycles
1	55°C	30 min	1
2	95°C	10 min	1
Hold	4°C	Indefinite	1

Example of calculations: Sample A has 0.4 million cells and sample B has 1.0 million cells. Cells are lysed with supplemented cell lysis buffer at 2,000 cells/µL cell lysis buffer.

In step **A.2**, aliquot (0.4 + 1.0) x 10^6/ 2,000 x 105% µL = 735 µL of cell lysis buffer (Cat #GQ400a), then add 735 µL x 10 µL/1 mL = 7.35 µL of cell lysis buffer enhancer (100x, Cat #GQ400b) to the aliquoted cell lysis buffer.

In step **A.3**, transfer 0.4 x 10^6/ 2,000 = 200 µL of supplemented cell lysis buffer to sample A, and 1.0 x 10^6/ 2,000 = 500 µL of supplemented cell lysis buffer to sample B.

B. RT-PCR setup

1. Prior to use, allow the primer set (Cat #7168-ps) to thaw to room temperature. Vortex briefly to mix well. Centrifuge the vials at 1,500x g for 15 seconds. Aliquot the primer set as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles. Keep on ice when thawed.

2. With the test samples, two control samples should be run concurrently: the purified or lysed non-infectious positive control (Cat #RU011-pos) as the positive control sample and H_2O (Cat #GQ100-4) as the No Template Control (NTC). Prepare one RT-PCR reaction for each control sample with #7168-ps primer set. Prepare 20 µl RT-PCR reactions as shown in Table 1.

Table 1.

Control sample (positive control or NTC)	2 µl
Primer set (Cat #7168-ps)	2 µl
RubyNStart one-step RT-PCR master mix, 4X (Cat #MB6058a)	5 µl
RubyNStart enhancer, 2X (Cat #MB6058b)	10 µl
Nuclease-free H_2O (Cat #GQ100-4)	1 µl
Total volume	***20 µl***

3. For each extracted DNA test sample, prepare one RT-PCR reaction with #7168-ps primer set. Prepare 20 µl RT-PCR reactions as shown in Table 2.

Table 2.

Test sample (concentration varies)	2 µl
Primer set (Cat #7168-ps)	2 µl
RubyNStart one-step RT-PCR master mix, 4X (Cat #MB6058a)	5 µl
RubyNStart enhancer, 2X (Cat #MB6058b)	10 µl
Nuclease-free H_2O (Cat #GQ100-4)	1 µl
Total volume	***20 µl***

4. Seal the RT-PCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.

5. Setup RT-PCR reactions as shown in Table 3.

Table 3. Instrument settings for RT-PCR reactions.

Step	Temperature	Time	Number of cycles
Reverse transcription	50°C	15 min	1
Taq DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	20 sec	38
Annealing	66°C	20 sec	
Extension	72°C	35sec	
Final extension	72°C	5 min	1
Hold	16°C	indefinite	1

Results Interpretation

Table 4. Interpretation of DHIV1 kit control sample test results.

Note: Primer dimers may form during RT-PCR reactions and show a band below 70 bp. To separate the primer dimer from the 85-bp human B2M band, let the gel run long enough on a 1.5% agarose gel.

Sample	HIV-1 175 bp	Human B2M 85 bp	Results Interpretation
RU011-pos	+	+	Expected
	-	-	RT-PCR failed
GQ100-4	-	-	Expected
	+	+	Reagent(s) contaminated

Table 5. Interpretation of DHIV1 kit target sample test results when control results are as expected.

HIV-1 175 bp	Human B2M 85 bp	Results Interpretation
+	+ or -	HIV-1 virus detected
-	+ or -	No HIV-1 virus detected