GeneCopoeia™
Expressway to Discovery

Protocol ● Lenti-Pac™ HIV qRT-PCR Titration Kits ● Cat. No. LT005 ● LT006

I. Description

The Lenti-Pac™ HIV qRT-PCR Titration Kits are designed for quick and simple titration of lentiviral particles. The kits include the RNA extraction reagent, qRT-PCR reagents and standard control.
The RNA genome of lentivirus is reverse-transcribed and quantified using qRT-PCR and SYBR® green technologies.

- Confirm the success of the lentiviral particle production
- Determine the virus copy number to ensure the efficiency of transduction and expression
- All reagents from viral RNA extraction to RT-PCR are included

II. Content and Storage

LT005 (20 RT reactions, 50 PCR reactions); LT006 (40 RT reactions, 100 PCR reactions)

Component	Volume	Concentration	Shipping	Storage
RNAzol® RT RNA extraction reagent	1 x 6 ml 2 x 6 ml		Ice pack	Room temperature Stable for at least 12 months
Linear polyacrylamide	1 x 75 µl 2 x 75 µl	1.5 mg/ml	Ice pack	4°C Stable for at least 12 months
DEPC water	1 x 1.25 ml 2 x 1.25 ml		Ice pack	Room temperature Stable for at least 12 months
DNase I (RNase-free)	1 x 20 µl 2 x 20 µl	2,000 units/ml	Ice pack	-20°C Stable for at least 12 months
DNase I buffer (10x)	1 x 50 µl 2 x 50 µl		Ice pack	-20°C Stable for at least 12 months
RNase inhibitor	1 x 20 µl 2 x 20 µl	40,000 units/ml	Ice pack	-20°C Stable for at least 12 months
Reverse transcription enzyme	1 x 20 µl 2 x 20 µl		Ice pack	-20°C Stable for at least 12 months
Reverse transcription buffer (10x)	1 x 40 µl 2 x 40 µl		Ice pack	-20°C Stable for at least 12 months
dNTP	1 x 20 µl 2 x 20 µl	25 mM each	Ice pack	-20°C Stable for at least 12 months
cDNA synthesis primer	1 x 100 µl 2 x 100 µl	4.0 µM	Ice pack	-20°C Stable for at least 12 months
qPCR standard (DNA)	1 x 20 µl 2 x 20 µl	$1x10^9$ copies/µl	Ice pack	-20°C Stable for at least 12 months
qPCR primer mix	1 x 100 µl 2 x 100 µl	2.5 µM each	Ice pack	-20°C Stable for at least 12 months
All-in-One™ qPCR mix (2x)	1 x 500 µl 2 x 500 µl		Ice pack	-20°C Stable for at least 12 months

Materials Required but Not Provided

Isopropanol or ethanol, molecular biology grade
75% ethanol, molecular biology grade (stored at 4°C)
Optional: Random primers (random hexadeoxynucleotides) at 500µg/ml (~250 µM) in TE buffer

Related Products

Lenti-Pac lentiviral packaging kits
Lenti-Pac 293Ta lentiviral packaging cell line
Lenti-Pac lentivirus concentration solution

III. Protocol

Please read the entire procedure carefully before starting the experiment.

RNA extraction

1. Add 0.25 ml of RNAzol® RT to a 1.5 ml tube containing 50 µl or 100 µl of purified lentiviral particle supernatant, or 10 µl of purified/concentrated lentiviral particles. Close the cap of the tube tightly. Invert the tube 10 times to lyse lentiviral particles and to solubilize proteins. Incubate for >15 minutes at room temperature.
2. Briefly spin the tube. Add 50 µl of water to the 50 µl crude sample tube, or 90 µl of water to the 10 µl purified/concentrated sample tube. The volume of water plus the volume of lentiviral sample equals 100 µl.
3. Centrifuge the homogenate for 10 minutes at 18,000×g at 20°C.
4. Carefully transfer the supernatant to a new 1.5 ml tube. Add linear polyacrylamide to a final concentration of ~15 µg/ml. For example, to 100 µl of supernatant add 1.0 µl of 1.5 mg/ml linear polyacrylamide stock solution.

   Note: Linear polyacrylamide is used as a co-precipitant to improve the recovery of RNA during alcohol precipitation. Co-precipitated polyacrylamide also helps visualize RNA pellet. Linear polyacrylamide does not interfere with the following enzyme digestion, reverse transcription or PCR reactions.
5. Add one volume of 100% isopropanol or 3 volumes of 100% ethanol. Invert the tube to mix well. Preferably store the tube at -20°C for over 4 hours or overnight.
6. Centrifuge for 20 minutes at 18,000×g at 10°C. Discard the supernatant.
7. Wash the pellet twice with 0.5 ml of 75% ethanol. Centrifuge for 5 minutes at 18,000×g at 10°C. Discard the supernatant.
8. Remove residual ethanol as completely as possible. Air-dry the pellet for 3 minutes at room temperature. Do not over-dry the pellet. Dissolve the RNA pellet with 50 µl of DEPC water.

DNase I digestion (to eliminate lentiviral expression plasmid contamination)

In a 1.5 ml tube set up the following reaction (total volume 25 µl):

DEPC water	1.5 µl
Lentiviral RNA	20.0 µl
DNase I buffer (10x)	2.5 µl
DNase I	1.0 µl

Incubate for 30-60 minutes at 37°C; then incubate for 10 minute at 75°C to inactivate DNase I.

Note: If the DNase I digestion step is omitted, a control QPCR reaction using non-reverse transcribed RNA sample as template should be included. This control reaction will determine the copy number of plasmid DNA from carryover. The RNA copy number is then determined by subtracting this number (without RT) from the reaction using cDNA (with RT) as template.

Reverse transcription

1. In a 0.2 or 0.5 ml tube set up the following reaction (total volume 20 µl):

(DNase I treated) RNA	10.0 µl
4.0 µM cDNA synthesis primer*	5.0 µl (final concentration 1.0 µM)

Mix well. Incubate for 5 minutes at 70°C. Place the tube on ice to cool the sample.

Note: Random primers (final concentration 10 µM in the reverse transcription mixture) can be used in place of HIV cDNA synthesis primer. It is not necessary to use both cDNA synthesis primer and random primers.

2. Add the following components to the tube:

10×Reverse Transcription Buffer	2.0 µl
25 mM dNTP	1.0 µl
RNase Inhibitor	1.0 µl
Reverse Transcriptase	1.0 µl

Spin briefly to mix. Incubate for 60 minutes at 37°C.

3. Heat for 10 minutes at 90°C. Use the product directly for QPCR analysis, or store the tube at -20°C.

QPCR analysis

1. Set up the following qPCR reaction (total volume 20 µl):

Water	6.0 µl
2x All-in-One™ Q-PCR Mix	10.0 µl
qPCR primer mix (2.5 µM each)	2.0 µl (final concentration 0.25 µM each)
DNA standard or cDNA sample or water	2.0 µl

Note:

- A master mix that includes all but the DNA standard/cDNA is recommended for all reactions.
- No-template control should be included.
- Suggested amount of DNA standard: 1×10^8, 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, and 1×10^2.

2. Start PCR reaction cycles.

The following setting is optimized for Bio-Rad iQ5 real time PCR detection system. You may need to fine tune the cycling conditions for the system you are using.

Cycle	Steps	Temperature	Duration	Read
1	denaturation	95 °C	10 min	off
	denaturation	95°C	10 sec	off
40	anneal	60 °C	20 sec	off
	extension	72°C	15 sec	on

Melting Curve analysis:

Temperature	Interval	Duration	Read
72-95°C	0.5 °C	6 sec/each	on
30°C		30 sec	off

3. Data analysis:

1) Determine the C_t values for each of the standard DNAs. Plot the C_t against the copy number (log scale) to generate a standard curve. The correlation coefficient of the standard curve should be above 0.99.

2) Determine the C_t values for the cDNA samples. Retrieve the copy number for the corresponding C_t value, or determine the copy number by using the standard curve generated in the previous step.

3) Multiply the above raw copy number by the **dilution factor** to get the copy number for the original sample (copies/ml).

$$\text{Dilution factor} = \frac{\text{RNA volume (µl)}}{\text{original sample volume (µl)}} \times \frac{\text{DNase reaction volume (µl)}}{\text{volume of RNA used in DNase reaction (µl)}} \times \frac{\text{RT reaction volume (µl)}}{\text{volume of RNA used in RT reaction (µl)}} \times \frac{1000\text{µl/ml}}{\text{volume added to PCR well (µl)}}$$

Note:

- RNA volume: The volume of the extracted RNA dissolved in water (50 µl by standard protocol)
- Original sample volume: The volume of lentiviral particles used for the RNA extraction
- DNase reaction volume: 25 µl by standard protocol
- Volume of RNA used in DNase reaction: 20 µl by standard protocol
- RT reaction volume: 20 µl by standard protocol
- Volume of RNA used in RT reaction: 10 µl by standard protocol
- Volume added to PCR well: 2 µl by standard protocol

4) Each lentiviral particle contains two copies of positive single-stranded RNA. So the number of lentiviral particles per one ml is half of the determined copy number.

IV. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of all Lenti-Pac™ qRT-PCR Titration Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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