ab289841 – qPCR Lentivirus Titer Kit

PCR based kit to detect Lentivirus in virus producing cell lines or purified viral preparations.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: http://www.abcam.com/ab289841

Storage and Stability

The kit should be stored at -20°C upon arrival. Avoid repeated freeze and thaw cycles. All reagents are stable for up to 12 months when stored properly at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
2X qPCR MasterMix	1.25 mL	-20°C
Primer Mix	200 µL	-20°C
Standard Control DNA	50 µL	-20°C
ROX Reference Dye	15 µL	-20°C
Nuclease-Free Water	2 x 1 mL	-20°C
Virus Lysis Buffer	2 x 800 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- qPCR Thermal Cycler
- PCR tubes
- 1X Phosphate Buffered Saline or DMEM

Assay Protocol

The qPCR Lentivirus Titer Kit is SYBR based and not probe based. So, the end user does not have to choose a reporter. Please select "SYBR Green Reagents" on the qPCR machine and set the passive reference to "ROX".

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type:

- No ROX equipment: Not needed
- Low ROX equipment: 1 µL/1.25 mL MasterMix.
- High ROX equipment: 11 µL/1.25 mL MasterMix

1. **Sample Preparation**: For purified high titer viral samples, dilute the virus samples to 10^6 IU/mL range with 1X Phosphate Buffered Saline or DMEM. For low viral titer samples, collect viral supernatant for direct qPCR.
2. **Viral Lysis**: Add 2 µL of the sample preparation (from Step 1) to 18 µL of Virus Lysis Buffer and incubate at room temperature (RT) for 5 min. Use the lysed sample for the reaction set up (in Step 4).
   ΔNote: The viral sample has been diluted 1/10, thus take this dilution factor into consideration when calculating the final titer.
3. **Standard Control DNA Dilutions**: Perform four, 10-fold serial dilutions of the Standard Control DNA by diluting 5 µL DNA into 45 µL Nuclease-free Water in each step. Dilutions 1:10 to 1:10,000 will be used for generating the Standard Curve.
4. **Set-up**: All reactions are recommended to be set-up on ice in duplicates.

Component	Volume
2X PCR MasterMix	10 µL
Primer Mix	2 µL
Sample, NTC, or Standard DNAs	2 µL
Nuclease-Free Water	6 µL

5. qPCR cycling conditions:

Step	Temperature	Duration	Cycle(s)
Reverse Transcription	42°C	20 min	1
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	15 sec	34
Annealing/Extension	62°C	1 min	34

Data Analysis:

Plot Ct value (Y-axis, linear scale) vs. Virus titer (X-axis, logarithmic scale). Generate a logarithmic regression using the four (4) Standard Control DNA dilutions to determine the unknown virus sample titer using y = mln(x) + b from the trendline equation. The R^2 Value should be > 0.95 to justify the proper assay setup. *Note to include the dilution factor of 10 plus additional diluting factor for purified viral samples in the final calculation.*

Virus titer (IU/mL) = $e^{(Ct - b)/m}$

Where:
m = the slope of the line
b = the y-intercept

Example: trendline equation is y = -1.349 ln(x) + 40.898; Ct of unknown sample = 16.98

Virus titer (IU/ml) = $e^{(16.98 - 40.898)/-1.349}$ = 5.01 x 10^7 IU/mL

Dilution	Virus Titer (IU/mL)
1:10	1 x 10^6
1:100	1 x 10^5
1:1,000	1 x 10^4
1:10,000	1 x 10^3

Technical Support

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