qPCR Lentivirus Titer Kit

Cat. No. LV900

Store at -20°C.

Product Description

abm's qPCR Lentivirus Titer Kit is a one-step assay which **employs a quick RNA extraction step that is followed by RT-qPCR**. Designed to deliver **high sensitivity and specificity**, the kit ensures minimal non-specific background and better overall performance compared to similar kits on the market. ROX reference dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments. MasterMix contains dye comparable to SYBR Green ™ and EvaGreen™.

Product Component	Quantity	Part No.
BlasTaq™ 2X qPCR Titer MasterMix	1.25 ml	P889-1
Primer Mix	100 rxn (200 µl)	LV900-A
Standard Control DNA	50 µl	LV900-B
Virus Lysis Buffer	800 µl	LV900-C
ROX Reference Dye	15 µl	P101
Nuclease-Free H_2O	2 x 1.0 ml	P100

Protocol

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 to 10^7 IU/ml range with 1X PBS or DMEM. For low viral titer samples, collect viral supernatant for direct qPCR set up. Contact us at technical@abmgood.com for tips on titering lentiviruses packaged with suspension cells.

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 for 3 min. Use the lysed sample for the reaction set up (in Step 4). Note: 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 five (5) 10-fold serial dilutions of the Standard Control DNA by diluting 5 µl DNA into 45 µl Nuclease-free H_2O in each step. Dilutions 1/100 to 1/100,000 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 qPCR MM	10 µl
Primer Mix	2 µl
Sample, NTC, or Standard DNAs	2 µl
Nuclease-free H_2O	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	30
Annealing/Extension	60°C	1 min	

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 $y = m\ln(x) + b$ from the trendline equation. The R^2 value should be > 0.95 to justify the proper assay setup. Note to include the dilution factor in the final calculation (i.e. if you diluted your purified viral sample 1/100 in Step 1 with 1/10 dilution from Step 2, then the titer of the unknown sample should be multiplied by a factor of 1000).

Virus titer (IU/ml) $= e^{(Ct-b)/m}$, where m is the slope of the line and b is the y-intercept.

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

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

Dilution	Virus Titer (IU/ml)
1/100	1×10^9
1/1,000	1×10^8
1/10,000	1×10^7
1/100,000	1×10^6

Download **abm**'s calculation file from the product page on our website.

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