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.

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

MasterMix contains dye comparable to SYBR Green™ and EvaGreen™. ROX Reference Dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments. *See **Rox Machine Compatibility** on our product page under the Documents tab on our website.*

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 10^6 IU/ml range (for best results) with 1X PBS or DMEM.
2. **Viral Lysis:** Add 2 µl of the sample preparation (from Step 1 or 2 µl of viral supernatant for low titer preparation) to 18 µl of Virus Lysis Buffer and incubate at room temperature for 5 minutes followed by a snap spin. Use the lysed sample for the reaction set up (in Step 4). Note: *The viral sample has been further diluted by another factor of 10, thus the total sample dilution factor should be included in when calculating the final titer, especially for purified virus sample titering.*
3. **Standard Control DNA Dilutions:** Perform four (4) 10-fold serial dilutions of the Standard Control DNA (5 µl DNA into 45 µl Nuclease-free H_2O). 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 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	34
Annealing/Extension	62°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 of 10 plus additional diluting factor for purified viral samples in the final calculation.*

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 $y = -1.408 \ln(x) + 37.42$; Ct of unknown sample = 17.16

Virus titer (IU/ml) = $e^{(17.16 - 37.42)/-1.408}$ = 1.77×10^6 IU/ml

Dilution	Virus Titer (IU/ml)
1/10	1×10^6
1/100	1×10^5
1/1,000	1×10^4
1/10,000	1×10^3

*Download the **qPCR Lentivirus Titer Calculation Form** from the product page under the Datasheet Tab on our website.*

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com