qPCR Retrovirus Titer Kit

Cat. No. G949

Store at -20°C.

Product Description

qPCR Retrovirus 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	Volume	Part No.
BlasTaq™ 2X qPCR Titer MasterMix	1.25 ml	P889-1
Primer Mix	100 rxn (200 µl)	G949-A
Standard Control DNA	50 µl	G949-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: 1.0 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 107 IU/ml range with 1X PBS or DMEM. For low viral titer samples, collect viral supernatant for direct qPCR set up.
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	Cycles
Reverse Transcription	42°C	20 min	1
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	15 sec	30
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 in the final calculation (i.e. if you diluted your purified viral sample 1/100 in Step 1 with 1/10 dilution in 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	$4x10^8$
1/1,000	$4x10^7$
1/10,000	$4x10^6$
1/100,000	$4x10^5$

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

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