Product Description

TribioScience's Lentivirus (LV) qPCR Titer Kit incorporates TribioScience FAST technology paradigm and probe Taqman qPCR technology for an accurate, one-step, and time-saving qPCR titer determination of lentivirus. The probe is labelled with Fluorescence FAM. The entire procedure, which does not require additional reverse transcription step, can be completed within 2 hours. With its time-saving 1-minute enzyme activation technology, specificity, and sensitivity, the performance of TribioScience's Lentivirus qPCR Titer Kit is unmatched by similar kits currently on the market.

Kit Content for 100RXN

Component	Volume	Part Number
FAST Lysis Buffer	1.0 mL	LV001
Fast qPCR mix	1.0 mL	LV002
Primer-Probe Mix	0.3 mL	LV003
LV Control DNA	30 µL	LV004
PCR Buffer	1.0 mL	LV005

Note: The probe is labelled with Fam.

Storage and Shelf-life

Store at -25ºC to -15ºC immediately upon arrival. Minimize the number of freeze-thaw cycles to ensure superior performance. The Kit is stable for one (1) year from the date of arrival.

Protocol

1. **Viral Sample Preparation**

A. **Purified viral particles:** Bring viral particles to 10^6 IU/ml with PBS.

B. **Virus producing cell cultures**: centrifuge cell culture medium at 2000g for 5 minutes to sediment suspended cells and cell debris. Use supernatant for the next step.

2. **Viral Lysis**

Add 2 µL of the sample preparation to 18 µL of FAST Lysis Buffer (1:10 dilution). Pipette gently up and down to mix and incubate at room temperature (23ºC) for 3 minutes. Use the lysed sample for the reaction set up in Step4. *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 Preparation**: The LV Control DNA is $1x10^8$ IU/mL. Perform 5 serial dilutions of the Standard Control DNA at 10-fold manner by diluting 2 µL Standard DNA into 18 µL PCR buffer in each concentration. Dilutions 1/10 to 1/100,000 will be used for generating the standard curve. The standard preparation is listed in table 1:

Table 1: Standard Preparation

Dilution	Virus Titer (IU/ml)
1/10	$1x10^7$
1/100	$1x10^6$
1/1000	$1x10^5$
1/10000	$1x10^4$
1/100000	$1x10^3$

4. **qPCR Preparation:** All reactions are set up on ice in duplications. The reaction volume is 20 µL. The details are listed in Table 2.

Table 2: qPCR Reaction Setup

Component	Volume (µL)
Fast qPCR mix	10.0
Primer-Probe mix	2.0
DNA Sample or Standard, NTC	2.0
PCR Buffer	6.0
Final Volume	20

Note: PCR Buffer is used as NTC.

5. **qPCR Running Parameters:** Program the qPCR instrument as Table 3, and Color Channel is FAM:

Table3: PCR Conditions

Purpose	Temperature (°C)	Time	Cycle(s)
Enzyme Activation	94	60S	1
Denaturation	94	10S	40
Priming/Extension	60	60S	

6. **Titer Calculation**

Plot Ct value (Y-axis, Linear scale) vs. Virus titer (X-axis, Logarithmic scale). Generate a logarithmic regression using the five (5) Standard Control DNA dilutions to determine the unknown virus sample titer using y= mIn(x) +b for 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 samples 1/100 in step 1 and 1/10/ in Step 2, then the titer of the unknown sample should be multiplied by a factor of 100x10).

Use the Ct values to calculate the viral titer of the viral sample with the following formula:

Titer of unknown sample (IU/ml) = $e^{(Ctx-b)/m}$, Where m is the slope of the line, and b is the y-intercept.

Example: Trendline equation is y= -1.349In(x) + 40.898; Ct if unknown sample = 16.98

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

Note: Remember to include the dilution factor in the calculation if dilutions of viral particles had been performed.