TransLv™ Lentivirus qPCR Titration Kit

Cat. No. FV201

Storage: at -20°C in the dark for one year

Description

TransLv™ Lentivirus qPCR Titration Kit provides a simple, rapid, sensitive and efficient method to determine the copy number of integrated proviral sequences in lentivirus transduced cells using qPCR method. Lentivirus titer can be calculated from integrated proviral copy number in the genome of the lentivirus transduction susceptible cells (such as HT1080, HEK-293T, *etc*.) by using the standard curve generated from qPCR.

- Simple operation and high sensitivity
- Higher accuracy enabled by internal reference gene
- Compatible with the second and third generation HIV-1 lentiviral packaging vectors
- Good linear relationship in the range of 10^3-10^8 copies/μl

Composition

Component	100 reactions
2×*TransLv*™ Lentivirus qPCR Titration SuperMix	1 ml
10×GC enhancer	200 μl
Provirus Gene Standards (P1-P6) (10^8-10^3 copies/μl)	30 μl each
Reference Gene Standards (R1-R6) (10^8-10^3 copies/μl)	30 μl each
Provirus Gene Primer Mix (5 μM)	100 μl
Reference Gene Primer Mix (5 μM)	100 μl
Passive Reference Dye（50×）	40 μl
Nuclease-free Water	1 ml

Procedures

Materials required but not included:

Product Name	Catalog
EasyPure® Genomic DNA Kit	TransGen, Cat. EE101
TransLv™ Lentivirus Precipitation Solution	TransGen, Cat. FV101

A. Transduction of target cells

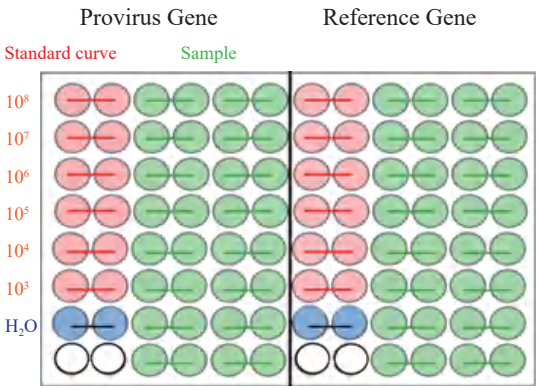
To obtain high efficiency of viral transduction, it is suggested that *TransLv*™ Lentivirus Precipitation Solution (TransGen, Cat. FV101) be used for lentiviral concentration after packaging. For lentiviral titer determination, susceptible cells (such as HT1080, HEK-293T, *etc.*) are recommended as target cells. Lentiviral transducedcells are harvested 48-72 hours after transduction for genomic DNA extraction.

B. Genomic DNA extraction

Isolate genomic DNA in lentiviral transduced cells and dilute it to the range of 5-100 ng/μl according to the isolation yield. It is recommended to try serial dilution of genomic DNA for the first use, to make sure thatthe copy number is in the range of 10^3-10^8 copies/μl. *EasyPure*® Genomic DNA Kit (TransGen, Cat. EE101) is recommended for genomic DNA extraction.

C. Amplification of proviral sequence

The extracted Genomic DNA and the two sets of standards were subjected to the same qPCR procedure, with at least two parallels per sample.



1) Reaction components (20 µl)

Component	Volume
Template	2 µl
Primer Mix (5 µM)	1 µl
2×*TransLv*™ Lentivirus qPCR Titration SuperMix	10 µl
10×GC Enhancer	2 µl
Passive Reference Dye (50×) (optional)	0.4 µl
Nuclease-free Water	Variable
Total Volume	20 µl

Passive Reference Dye for use on the following instruments:

- Passive Reference Dye I (50×)
  ABI Prism 7000/7300/7700/7900, ABI Step One, ABI Step One Plus, ABI 7900HT, ABI 7900HT Fast
- Passive Reference Dye II (50×)
  ABI Prism 7500, ABI Prism 7500 Fast, ABI QuantStudio Dx/3/5, ABI QuantStudio 6/7/12K Flex, ABI ViiA 7, Stratagene Mx3000P/Mx3005P/Mx4000
- No Passive Reference Dye
  Roche LightCycler 480, Roche Light Cycler 96, MJ Research Chromo4, MJ Research Opticon 2, Takara TP-800, Bio-Rad iCycler iQ, Bio-Rad iCycler iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Scientific Pikoreal 96, Qiagen Corbett Rotor-Gene 6000, Qiagen Corbett Rotor-Gene G, Qiagen Corbett Rotor-Gene Q, Qiagen Corbett Rotor-Gene 3000, Mastercycler ep realplex

2) Thermal cycling conditions

50℃	2 min	
95℃	5 min	
95℃	15 sec	35-40 cycles
60℃	30 sec	
Dissociation Stage		

D. Data analysis

1) Determine the copy number corresponding to the sample genome based on the standard curve.

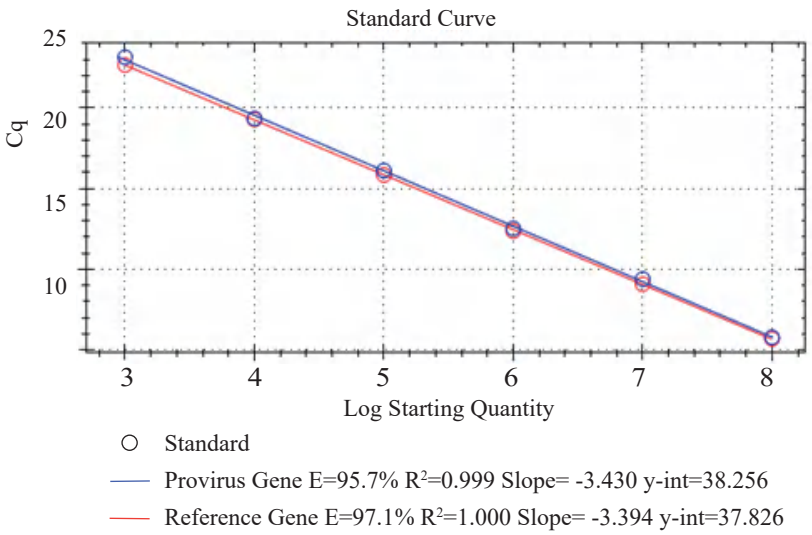
2) Calculate the titer of the lentivirus according to the following formula.

$$\text{Integrated lentiviral copies per cell} = \frac{\text{Quantity mean of the Provirus Gene}}{\text{Quantity mean of the Reference Gene}} \times 2$$

$$\text{Lentiviral titer (IU/ml)} = \frac{\text{number of cells inoculated in each well} \times \text{number of integrated lentiviral copies per cell}}{\text{volume of lentiviral solution used (ml)}}$$

Example

1. HEK-293T cells (1×10^5 cells/well, 12-well plate) were transduced with 0.5 μl, 5 μl and 50 μl of concentrated lentivirus. 72 hours after transduction, genomic DNA was isolated and qPCR amplification was carried out according to the above procedures.



A strong linear correlation between the Ct values of the serial dilutions and copy number (log scale) was observed in the standard curve. Amplification efficiency was 95.7%, $R^2 = 0.999$ for Provirus Gene (Blue); amplification efficiency was 97.1%, $R^2 = 1.000$ for Provirus Gene (Red).

2. Calculate the lentivirus titer based on the amplification curve

Volume (μl)	0.5	5	50
Quantity mean of Provirus Gene	4766.36	44434.48	856284.42
Quantity mean of Reference Gene	34936.88	30825.53	28223.79
Lentivirus copies of per cell	0.27	2.88	60.68
IU/ml	5.46E+07	5.75E+07	1.20 E+08
Mean (IU/ml)	7.74E+07		

Notes

- Avoid cross-contamination in qPCR reaction.
- In order to ensure reliable experimental data, at least 2 or more parallels are required.
- For your health, please wear gloves at all times and follow biosafety guidelines.

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