

**Description**

The Lentivirus RT-qPCR Titration Kit is designed to provide a fast, sensitive, and reliable method to quantify lentiviral particle titers by measuring encapsidated viral RNA genomes using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR). The kit includes enough reagents for RNA extraction, plasmid DNA or residual genomic DNA removal, reverse transcription, and qPCR amplification of the conserved lentiviral element for 100 reactions, including standard curve and controls.

This kit is ideal for researchers producing lentiviruses for gene delivery, gene editing, or cell engineering applications who require accurate titers. The Lentivirus RT-qPCR Titration Kit enables precise measurement of the lentiviral RNA copy numbers present in cell culture supernatants. It includes ROX™ (carboxyrhodamine), a passive fluorescence reference dye that allows for signal normalization. By comparing Ct (cycle threshold, also known as cycle quantification, Cq) values to a standard curve generated from a known Standard DNA control, researchers can accurately determine the genomic copy number (GC/μl) of their lentiviral preparations.

**Background**

Lentiviral vectors are widely used in gene delivery applications due to their ability to integrate into the host genome, transduce both dividing and non-dividing cells, and to mediate long-term expression of transgenes. Accurate quantification of lentiviral particles is essential for achieving reproducible transduction efficiencies, optimizing experimental conditions, and ensuring the safety and consistency of gene therapy products. Traditional methods for measuring lentiviral titers, such as p24-based ELISA or functional assays based on transgene expression are either indirect, time-consuming, or subject to biological variability. In contrast, Reverse Transcription quantitative PCR (RT-qPCR) offers a rapid, sensitive, and reproducible approach to directly quantify the number of viral genome copies. A qPCR-based titration method is ideal for both *in vitro* and *in vivo* applications and is compatible with various qPCR platforms. It provides a reliable, standardized alternative to biological titration methods, facilitating quality control and scalability in lentivirus production workflows, making it a crucial tool in cell and gene therapy.

**Application**

- Lentivirus titration for transduction optimization experiments.
- Quality control during lentiviruses production.
- Verification of lot-to-lot consistency.
- Quantifying genome-containing particles for downstream *in vivo* or *in vitro* use.

**Supplied Materials**

Catalog #	Name	Amount	Storage
83574	Viral RNA Extraction Buffer	800 μl	-20°C
83575	DNase I Reaction Mix	800 μl	-20°C
83576	Standard DNA	30 μl	-20°C
83577	2x qPCR Master Mix	1000 μl	-20°C
83578	Primer Mix	200 μl	-20°C
83579	RT Master Mix	40 μl	-20°C
83580	ROX™ Reference Dye	15 μl	20°C
83581	Nuclease-Free Water	2 x 1000 μl	Room Temp

### Materials Required but Not Supplied

- Adjustable micropipette and RNase, DNase, DNA, and pyrogen-free, sterile filter tips
- qPCR instrument
- PCR tubes or plate
- Lentivirus sample of interest

### Storage Conditions



Components are shipped in dry ice and should be stored at the recommended temperature for long term storage. The components maintain their stability and performance up to 5 freeze-thaw cycles.

### BioSafety



This product is for research purposes only and not for human or therapeutic use. Overall, this product should be considered hazardous and harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly. BPS Bioscience recommends following all local, federal, state, and institutional regulations and using all appropriate safety precautions.

### Assay Protocol

- We recommend all reactions are set-up on ice, in duplicate.
- The assay should include “Standard DNA” (Positive Control), “NRT” (No Reverse Transcriptase Control), “NTC” (No Template Control) and “Lentiviruses Sample” conditions.
- The NTC and NRT conditions are essential negative controls to ensure accurate and reliable results. The NTC helps to confirm that any detected signal in your sample is truly derived from viral RNA and not from background contamination. The NRT identifies any DNA contamination in your RNA preparation. Including both controls ensures your lentiviral titer reflects the true viral RNA levels and helps avoid false-positive results due to contamination or DNA carryover.
- The viral sample has been diluted **100-fold**. This sample dilution factor should be included when calculating the final titer.
- The melt curve analysis is an essential step in qPCR, especially when using SYBR™ Green dye-based detection, as in this kit. SYBR™ Green binds to any double-stranded DNA and not just your target. So, the presence of primer-dimers or non-specific products will also generate fluorescence. A melt curve helps to confirm whether a single, specific product was amplified.
- The suggested quantity of ROX™ Reference Dye to include in the Master Mix differs depending on the type of qPCR instrument:
  - For equipment that does not require ROX: no dye is needed.
  - For low ROX instruments: use 1 µl of ROX Reference Dye per 1000 µl of Master Mix.
  - For high ROX instruments: add 10 µl of ROX Reference Dye per 1000 µl of Master Mix.

### Lysis of Viral Particles

1. Mix 2 µl of the lentiviruses sample with 18 µl of Viral RNA Extraction Buffer.

2. Incubate at Room Temperature (RT) for 5 minutes to release the viral RNA.

### DNase I Treatment

1. Mix 2  $\mu$ l of viral RNA prepared above with 18  $\mu$ l of DNase I Reaction Mix.
2. Incubate at 37°C for 30 minutes to remove plasmid DNA or residual genomic DNA.

### Standard DNA Dilutions

1. Prepare a serial dilution of Standard DNA ( $1 \times 10^7$  GC/ $\mu$ l stock) according to the dilution scheme shown in the table below:

Dilution Series	Volume of DNA Standard ( $\mu$ l)	Volume of Nuclease-Free Water ( $\mu$ l)	Dilution factor	GC/ $\mu$ l
Dilution 1	2 $\mu$ l	18 $\mu$ l	10 X	$1 \times 10^6$ GC/ $\mu$ l
Dilution 2	2 $\mu$ l of Dilution 1	18 $\mu$ l	10 X	$1 \times 10^5$ GC/ $\mu$ l
Dilution 3	2 $\mu$ l of Dilution 2	18 $\mu$ l	10 X	$1 \times 10^4$ GC/ $\mu$ l
Dilution 4	2 $\mu$ l of Dilution 3	18 $\mu$ l	10 X	$1 \times 10^3$ GC/ $\mu$ l
Dilution 5	2 $\mu$ l of Dilution 4	18 $\mu$ l	10 X	$1 \times 10^2$ GC/ $\mu$ l

### RT- qPCR Set-Up

1. Prepare the following reactions on ice in duplicate, as described in the table below:

Component	Standard DNA	NTC	NRT	Lentiviruses Sample
2X qPCR Master Mix	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
Primer Mix	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l
RT Master Mix	-	-	-	1 $\mu$ l
Diluted Standard DNA	2 $\mu$ l	-	-	-
Lentiviruses Sample	-	-	2 $\mu$ l	2 $\mu$ l
Nuclease-Free Water	6 $\mu$ l	8 $\mu$ l	6 $\mu$ l	5 $\mu$ l
<b>Total</b>	<b>20 <math>\mu</math>l</b>	<b>20 <math>\mu</math>l</b>	<b>20 <math>\mu</math>l</b>	<b>20 <math>\mu</math>l</b>

*Note: The viral sample has been diluted **100-fold**. This sample dilution factor should be included when calculating the final titer.*

2. Set up the RT-qPCR cycling conditions as follows:

Step	Temperature (°C)	Duration	Cycles
Reverse Transcription	60°C	10 minutes	1
Enzyme Inactivation/Initial Denaturation	95°C	3 minutes	1
Denaturation	95°C	15 seconds	35
Annealing/Extension	60°C	30 seconds	
Melt Curve	60°C to 95°C, increments of 0.5°C		

### Melt Curve Analysis

- Plot Ct value (Y-axis, linear scale) as a function of Virus titer (X-axis, logarithmic scale) for the “Standard DNA” control.

Apply logarithmic regression to determine the unknown virus sample titer using the equation  $y = mx + b$  from the trendline equation:

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

For example: if the trendline equation is  $y = -1.298 \ln(x) + 40.978$ ; Ct of unknown sample = 17.08, the viral titer is (GC/μl) =  $e^{(17.08 - 40.978)/-1.298} = 9.91 \times 10^7$  GC/μl.

*Note: The  $R^2$  value should be > 0.99.*

- If the NRT control gives a copy number value during analysis, subtract that value directly from the values to correct for any DNA contamination.

*Notes: If you prefer to indicate virus titer in GC/ml, multiply by 1,000 (Standard DNA is typically quantified in GC/μl). GC/ml values are usually 10–100x higher than IU/ml, since not all viral particles are infectious. For example, if a lentivirus preparation has  $1 \times 10^9$  GC/ml (as determined by qPCR), and  $1 \times 10^7$  IU/ml (as determined by infecting HEK293 cells), it means only 1% of the particles are functionally infectious.*

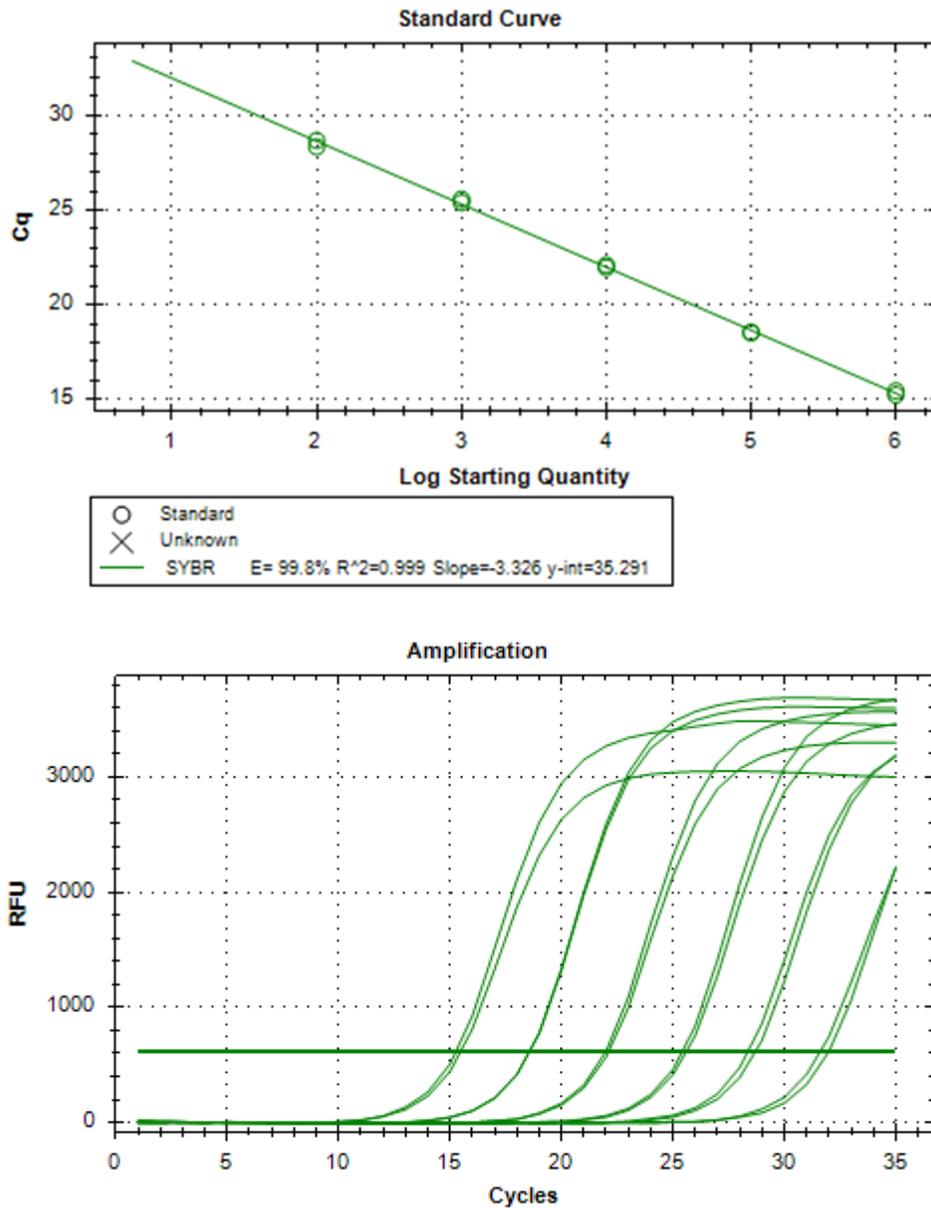


Figure 1: Standard curve and amplification curves generated with the Standard DNA.

Standard curve generated using serial dilutions of the Standard DNA for lentiviral genome quantification by RT-qPCR. The plot shows a strong linear relationship between the quantification cycle (Cq, also known as Ct) and the log of the standard DNA starting quantity, with an efficiency (E) of 99.8%,  $R^2 = 0.999$ , and a slope of -3.326. These values indicate high amplification efficiency and reliability of the standard curve for calculating genome copy numbers in unknown samples.

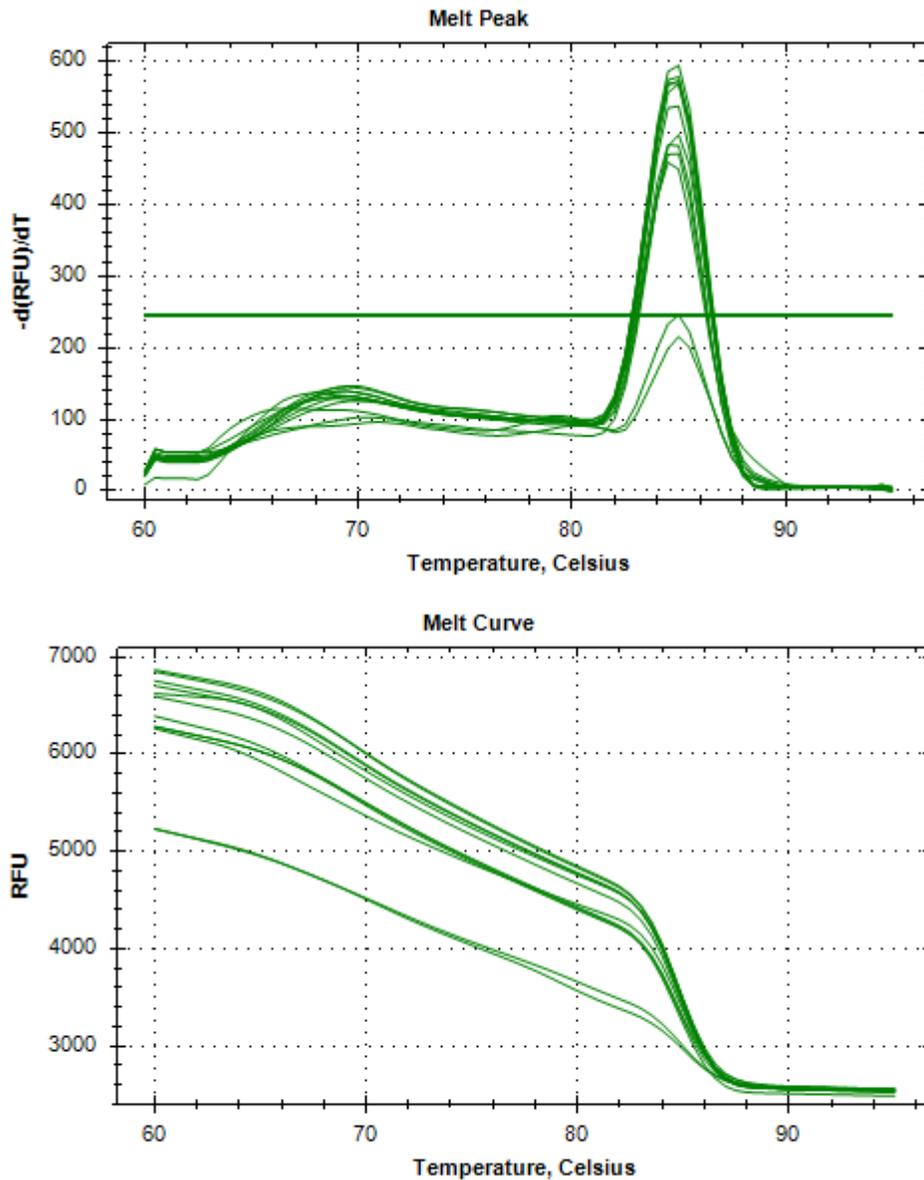
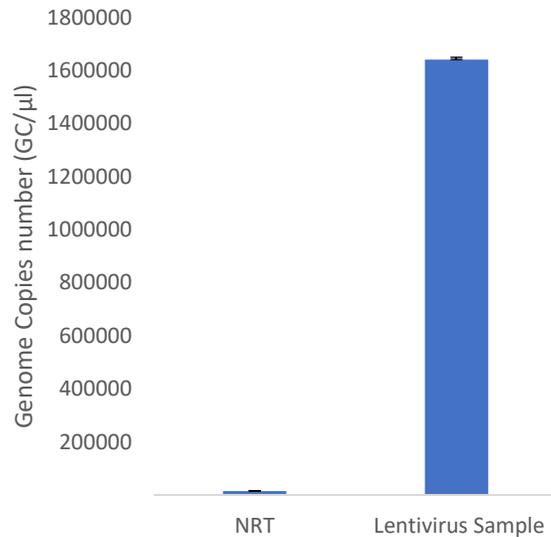


Figure 2. Melt curve analysis of qPCR products.

The melt curve was generated by gradually increasing the temperature from 60 °C to 95 °C while monitoring fluorescence (RFU). A sharp decrease in fluorescence between 80 °C and 90 °C indicates the melting temperature ( $T_m$ ) of the specific PCR products. The presence of a single melting transition suggests specificity of amplification with minimal primer-dimer or nonspecific products.

## Validation Data



*Figure 3: Lentiviral genome titer determination by RT-qPCR using Lentivirus RT-qPCR Titration Kit and including the NRT Control.*

The lentiviral genome titer of a lentiviral sample was determined with RT-qPCR using Lentivirus RT-qPCR Titration Kit and including the NRT Control. The bar graph shows the lentiviral genome copy number (GC/μl). The Lentivirus Sample displays a titer of approximately  $1.6 \times 10^7$  GC/μl. The NRT control, which omits reverse transcriptase to detect DNA contamination, shows minimal amplification, indicating negligible DNA carryover. This confirms that the measured signal in the lentivirus sample is primarily from viral RNA genomes. Error bars represent standard deviations from technical replicates.

*Data are representative.*

## Troubleshooting Guide

Visit [bpsbioscience.com/lentivirus-faq](https://bpsbioscience.com/lentivirus-faq) for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

## Related Products

Products	Catalog #	Size
AAV ONE-Extract™ Solution	78585	15-150 preps
AAV qPCR Titration Kit	82812	1 Kit
YFP (Topaz) Lentivirus	79989	500 μl x 2
RFP Lentivirus	78347-P	500 μl x 2
eGFP Lentivirus (Inducible TET On)	78629	500 μl x 2

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