Description

The Replication-Competent Lentivirus (RCL) qPCR Detection Kit is designed to provide a fast (less than 4 hours), sensitive (detects as low as 10 copies/µl), reliable and specific method to detect RCL contamination in both cell culture supernatants and transduced cells by quantitative Polymerase Chain Reaction (qPCR). The kit targets sequences derived from HIV-1 VSV-G genes that are typically retained in helper packaging systems and are absent in lentiviral vectors but would be present if RCL were generated. The kit includes enough reagents for 100 reactions, including standard curve and controls.

Background

RCL (Replication-Competent Lentivirus) detection is a critical measure in gene therapy and cell therapy workflows that utilize lentiviral vectors. Lentiviruses are widely used as gene delivery vehicles due to their ability to stably integrate genetic material into both dividing and non-dividing cells. However, during the packaging and production process, there is a low but significant risk of generating RCLs through recombination events. The presence of RCL poses serious biosafety concerns, including the potential for uncontrolled viral replication and insertional mutagenesis in transduced cells.

Application

- Test for RCL during cell line or CAR-T cell generation.

Supplied Materials

Catalog #	Name	Amount	Storage
83577	2x qPCR Master Mix	1000 µl	-20°C
83593	Standard RCL DNA	30 µl	-20°C
83592	Primer Mix	200 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
- Method to quantify DNA (e.g., Nanodrop or Qubit)
- qPCR instrument
- PCR tubes or plate
- 0.45 µm syringe filter
- viral DNA extraction kit

Storage Conditions

Components are shipped in dry ice and should be stored at the recommended temperature for long term storage. This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

BPS Bioscience

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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 triplicate.
- The assay should include "Standard RCL DNA" (Positive Control), "NCS" (Negative Control Solution), "NTC" (No Template Control), "ERC" (Extraction Recovery Control) and "Test Sample" conditions.
- In RCL detection the use of an NCS is a crucial part of quality control of qPCR assays. It helps ensure that any signal detected in test samples is due to actual RCL presence and not due to contamination or assay error. Never use water alone as the NCS, as it will not reflect the complex composition of cell culture media, and extraction process.
- NCS samples should be processed in parallel with the test samples, at all steps.
- ERC is used to ensure that your DNA extraction and qPCR are working properly and that no loss of DNA occurred.
- 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.

Standard RCL DNA Dilution Preparation

1. Prepare a serial dilution of Standard RCL DNA (stock concentration: 1 x 10^6 copies/ µl), as described in the table below:

Dilution Series	Volume of RCL Standard DNA (µl)	Volume of Nuclease-Free Water (µl)	Dilution factor	copies/ µl
Dilution 1	2 µl	18 µl	10 X	1 x 10^5 copies/µl
Dilution 2	2 µl of Dilution 1	18 µl	10 X	1 x 10^4 copies/µl
Dilution 3	2 µl of Dilution 2	18 µl	10 X	1 x 10^3 copies/µl
Dilution 4	2 µl of Dilution 3	18 µl	10 X	1 x 10^2 copies/µl
Dilution 5	2 µl of Dilution 4	18 µl	10 X	1 x 10^1 copies/µl

Test Sample and NCS Preparation

A. From Cell Culture Supernatant

1. Collect 1 ml of cell culture supernatant from untransduced / uninfected cells (i.e. the cell culture supernatant from the cell line used for vector production) for the NCS.
2. Collect 1 ml of cell culture supernatant from transduced / infected cells for the Test Sample.
3. Filter through a 0.45 µm syringe filter to remove cells or debris.
4. Use a viral DNA extraction kit to extract DNA from the NCS sample in parallel with the test samples.
5. Elute DNA with 50 µl nuclease-free water.

Note: 2 µl of the resulting DNA can then be amplified and analyzed by qPCR.

B. From Cell Pellets of Transduced Cells

1. Collect 1×10^6 cells (from ~80–100% confluent well of 6-well plate or T25 flask).
2. Centrifuge at 300 *× g* for 5 minutes.
3. Discard the supernatant and resuspend the cell pellet in 200 µl of PBS (phosphate buffer saline).
4. Use a viral DNA extraction kit to extract DNA.
5. Elute DNA in 50 µl of nuclease-free water.

Note: 2 µl (100 ng to 500 ng of genomic DNA per reaction) of the resulting DNA can then be amplified and analyzed by qPCR.

ERC Preparation

1. Add 1 ml of cell culture supernatant or a cell pellet from 1×10^6 cells of the test sample into a clean tube.
2. Add 2 µl of Dilution 3 of Standard RCL DNA (1×10^3 copies/µl).
3. Mix well and label this sample as ERC.

Note: Prepare an ERC for each test sample to monitor extraction and amplification efficiency throughout the process.

qPCR Set-Up

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

Component	Standard DNA	NTC	NCS	ERC	Test Sample
2x qPCR Master Mix	10 µl	10 µl	10 µl	10 µl	10 µl
Primer Mix	2 µl	2 µl	2 µl	2 µl	2 µl
Nuclease-Free Water	6 µl	8 µl	6 µl	6 µl	6 µl
Diluted Standard RCL DNA	2 µl	-		-	-
Test Sample	-	-	-	-	2 µl
NCS	-	-	2 µl	-	-
ERC	-	-	-	2 µl	-
Total	**20 µl**	**20 µl**		**20 µl**	**20 µl**

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

Step	Temperature (°C)	Duration	Cycles
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	-	-

Data Analysis

1. The system will automatically set the threshold in the Amplification Plot panel during analysis.

2. If the default threshold is too close to the baseline, leading to significant variations in Cq (cycle quantification) values between replicate wells, manually adjust the threshold to an appropriate level. After adjustment, use the Multicomponent Plot to assess whether the amplification curves appear normal.

3. Determine the spike recovery rate using the test results from the Test Sample and the spiked sample (ERC). The formula for calculating spike recovery is as follows:

   Spike Recovery Rate (%) = [(Measured RCL in ERC – Measured RCL from Test Sample) / (Amount of Standard RCL DNA)] × 100

   For example: if the measure of ERC sample prepared as described above (with 2 µl of Dilution 3) is 2,040 copies and the Test Sample reads a value of 10 copies, the Spike Recovery Rate is [(2040 - 10) / 2000] × 100 = 101.5%.

Note: Assay recovery should be in the 80-120% range. A lower value may indicate inability to read a real RCT, while a higher value may indicate contamination.

4. The NTC should either be N/A or display a Cq value that is higher than the mean Cq value of the lowest standard concentration on the curve.

5. **Negative** RCL Result: A sample is considered negative or below the kit detection level of RCL if no Cq value is detected in all replicate wells, or if only one well shows a Cq value and that value is greater than the Cq value corresponding to the Lower Limit of Quantification (LLOQ) (10 copies per reaction).

6. **Positive** RCL Result: A sample is considered positive if at least two replicate wells show a detected copy number of ⩾10 copies per reaction.

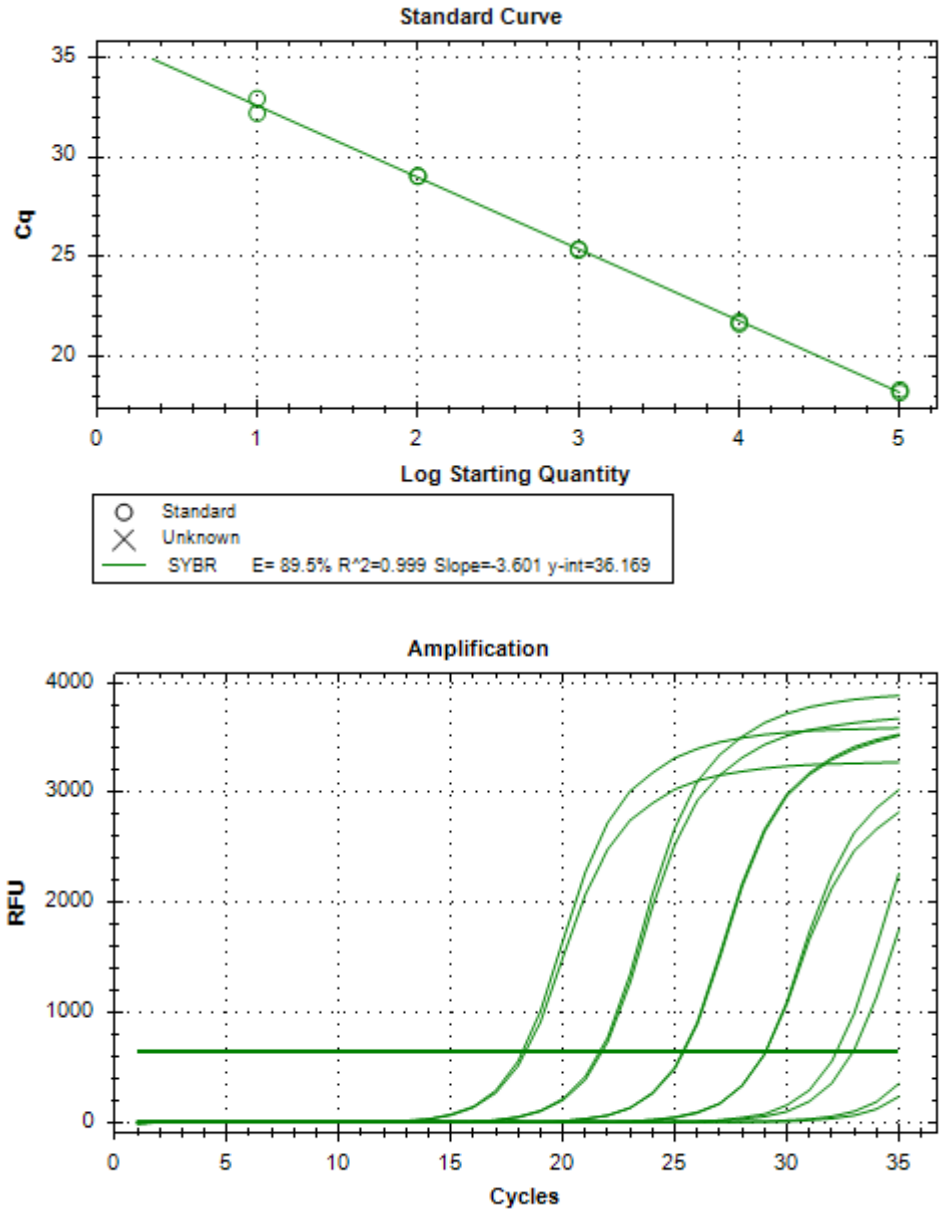

Figure 1: Standard curve and amplification curves generated with the Standard RCL DNA.
Standard curve generated using serial dilutions of the Standard RCL DNA by qPCR. The plot shows the quantification cycle (Cq) values plotted against the logarithm of the DNA quantity. The green line represents the best-fit linear regression of the standard data points (circles), with an amplification efficiency (E) of 89.5%, coefficient of determination (R^2) of 0.999, slope of –3.601. The data indicates a strong linear relationship and high reaction efficiency.

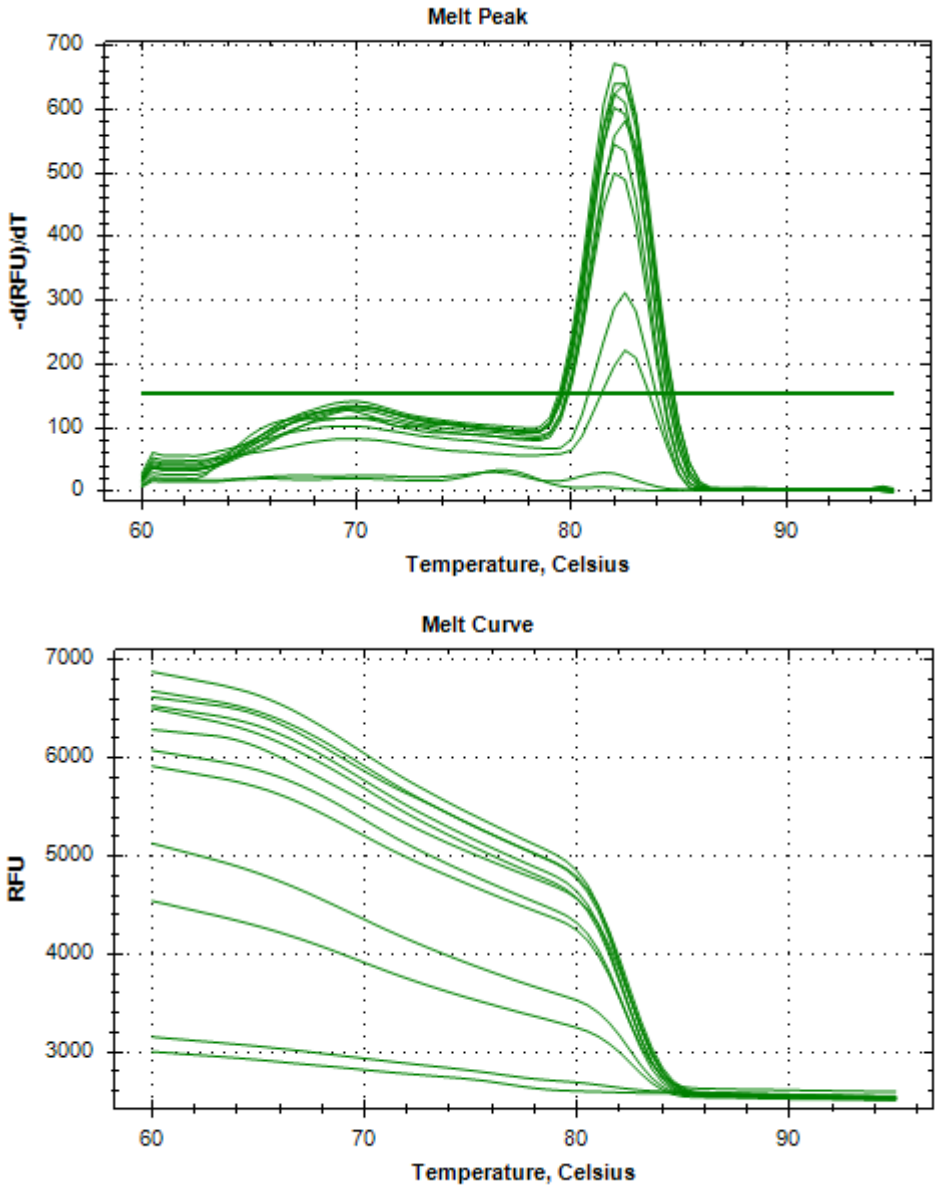

Figure 2. Melt curve analysis of qPCR products.
The melt curve was generated by gradually increasing the temperature from 60 °C to 95 °C while monitoring fluorescence (RFU). A sharp decrease in fluorescence between 80 °C and 90 °C indicates the melting temperature (Tm) of the specific PCR products. The presence of a single melting transition suggests specificity of amplification with minimal primer-dimer or nonspecific products.

Validation Data

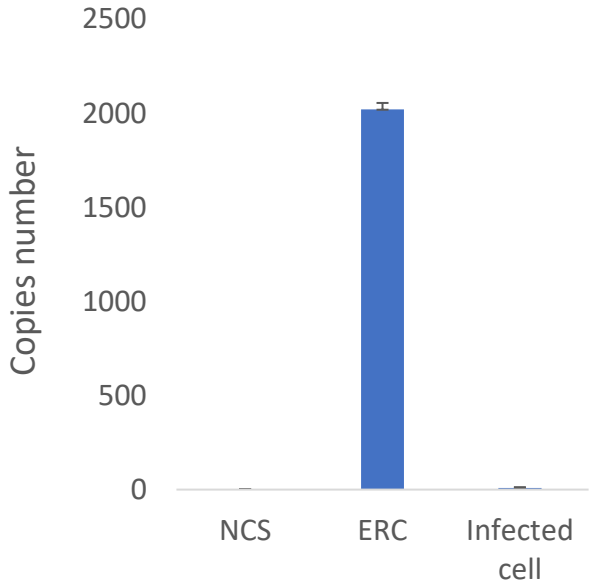

Figure 3: Quantitative analysis of VSV-G copies in test samples derived from cell culture supernatant of infected cells using the Replication-Competent Lentivirus (RCL) qPCR Detection Kit. The RCL of a cell culture supernatant was determined with qPCR using Replication-Competent Lentivirus (RCL) qPCR Detection Kit and including NCS (Negative Control Solution) and ERC (Extraction Recovery Control). A strong signal was observed in the spiked ERC sample, confirming the assay performance. Minimal or undetectable levels were found in both NCS and infected cell samples, indicating no evidence of RCL contamination. Error bars represent standard deviation from replicate reactions.

Data shown is representative.

Troubleshooting Guide

Visit 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*
Lentivirus RT-qPCR Titration Kit	83547	1 Kit
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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