Cat.: BP-QN10-100

RCL Gene Copy Number Detection Kit (qPCR) V01

Package Specification

100 Reactions / Kit

Intended Use

This kit is used for the quantitative detection of Replication-Competent Lentivirus (RCL) in cell therapy products and gene therapy products produced by lentiviral vectors.

Product Introduction

This kit adopts the PCR-fluorescent probe method, with primers and probes designed for the VSV-G gene. It uses ready-to-use calibrators prepared by serial dilution after digital PCR quantification, enabling accurate quantification of RCL copy numbers in cell and gene therapy products.

The kit contains an internal control to monitor abnormalities in nucleic acid extraction and amplification, preventing false-negative results. It also includes UDG enzyme to prevent carry-over contamination of amplification products.

Main Components

Components	Specification
RCL qPCR MIX 1	0.9 mL×2 Tubes
RCL qPCR MIX 2	0.2 mL × 1 Tube
RCL Negative Control	1.0 mL×2 Tubes
RCL Calibrator ST1	0.5 mL × 1 Tube (1×10^1 copies/μL)
RCL Calibrator ST2	0.5 mL × 1 Tube (1×10^2 copies/μL)
RCL Calibrator ST3	0.5 mL × 1 Tube (1×10^3 copies/μL)
RCL Calibrator ST4	0.5 mL × 1 Tube (1×10^4 copies/μL)
RCL Calibrator ST5	0.5 mL × 1 Tube (1×10^5 copies/μL)
RCL Calibrator ST6	0.5 mL × 1 Tube (1×10^6 copies/μL)

Notes

Components in different batch numbers of reagent kits are not interchangeable.

Reagents required for experimental procedures but not included in the kit: nucleic acid extraction or purification kit.

Storage conditions and shelf life

1. Store in the dark at ≤ -20℃ with a validity period of 24 months.
2. Avoid repeated freeze-thaw cycles; the number of repeated freeze-thaw shall not exceed 10 times.
3. The product validity period and expiration date are indicated on the product label.

Applicable Instruments

Including but not limited to the following models: SLAN-96P, SLAN-96S Automatic Medical PCR Analysis System; ABI7500, ABI QuantStudio™ 5 Real-Time Fluorescent Quantitative PCR Instrument; Roche LightCycler 480 Fluorescent Quantitative PCR Instrument; Bio-Rad CFX96 Quantitative PCR Instrument.

Detection method

Take RCL qPCR MIX1, RCL qPCR MIX2, RCL Negative Control, RCL Calibrators ST1–ST6 out of the kit, thaw at room temperature, mix thoroughly by vortexing, and centrifuge briefly before use.

1. Preparation of Extraction and Recovery Control (ERC)

Set the RCL spiking concentration in ERC as needed (take preparation of ERC with 1×10^6 copies as an example):

1)Add 100 μL of the sample into a clean 1.5 mL centrifuge tube;

2) Add 10 μL of ST5, mix well and centrifuge briefly, and label it as sample ERC.

3) Extract and purify nucleic acid from Sample ERC together with test samples in the same run.

2. Preparation of Reaction Mixture

2.1 Calculate the required number of PCR reaction solutions according to the number of test samples, and it is generally recommended to set 3 replicates per sample.

Number of PCR reactions = (6-concentration gradient standard curve + 1 No-Template Control NTC + 1 Negative Control NCS + test samples + test samples ERC) × 3 + 1 well for loss. Dispense RCL qPCR MIX into 96-well PCR plates or PCR 8-

strip tubes at 20 μL per reaction (refer to the table in Section 2.2 for RCL qPCR MIX preparation).

2.2 RCL qPCR MIX preparation method:

Component	Single volume (μL)
RCL qPCR MIX 1	18 μL
RCL qPCR MIX 2	2 μL
Total	20 μL

2.3 Example of sample addition for each reaction well:

Component	Sample Volume
Standard Curve	20 μL RCL qPCR MIX + 20 μL ST1/ST2/ST3/ST4/ST5/ST6
NTC	20 μL RCL qPCR MIX + 20 μL Negative Control
NCS	20 μL RCL qPCR MIX + 20 μL NCS Purified Solution
Test Sample	20 μL RCL qPCR MIX + 20 μL Test Sample Purified Solution
Sample ERC	20 μL RCL qPCR MIX + 20 μL Sample ERC Purified Solution

3.Nucleic Acid Extraction of Samples

Follow the instruction manual of the nucleic acid extraction or purification kit for the operation steps, with a sample volume of 100μL.

4.Fluorescent PCR Reaction

4.1 Add nucleic acid into RCL qPCR MIX according to Section 2.3, seal reaction tube caps or cover 96-well PCR plate with optical film, mix well, centrifuge briefly, and transfer to fluorescent PCR instrument.

4.2 Run the following program on the fluorescent PCR instrument:

Steps	Conditions	Cycles
UDG Treatment	25°C: 5 minutes	1
reverse transcription	50°C: 30 minutes	1
Pre-denaturation	95°C: 3 minutes	1
PCR Amplification	95°C: 10 seconds, 60°C (fluorescence collection): 30 seconds	45

Fluorescence channels: FAM (for RCL gene), ROX (for internal control).For ABI instruments: set reference dye to none.

5.Result Determination

5.1 Threshold Setting

Adjust threshold based on instrument noise. Set to the mean fluorescence of cycles 3–15 plus 10× standard deviation, or to the maximum fluorescence of negative controls, above background fluctuation.

5.2 Internal Control Validation

For negative results, the Ct value of the Internal control should be ≤35; For positive results, the Internal control may have no value or poor value due to competitive inhibition.

The internal control is a single-copy human gene. For samples that do not contain the human genome or have a low content of the human genome, the internal control will not provide a value or the provided value will be poor.

5.3 Acceptance Criteria

1) NTC and NCS show no Ct or mean Ct > mean Ct of the lowest calibrator.

2) Standard curve $R^2 \geq 0.98$, slope between −3.1 and −3.8.

3) Recovery of spiked samples (if applicable): 50% – 150%.

6.Result Analysis

6.1 Taking SLAN-96P as an Example:

1) If the threshold needs to be adjusted, set the threshold to an appropriate level in the "Parameter Settings" of the "Experiment Analysis" panel;

2) In “Plate Editor”, set calibrators as “Standard” and assign values of 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, 1×10^1 copies/μL in “Attribute” column, and name them ST6, ST5, ST4, ST3, ST2, ST1 in “Sample Name” column.

3) View slope, intercept, R^2, and amplification efficiency in “Standard Curve”.

4) Read concentration (copies/μL) in “Concentration” column of “Reaction Well Information Table” panel of “Experimental

Analysis". Calculate spiking recovery based on test sample and Sample ERC results; recovery shall be 50%–150%.

6.2 Taking ABI 7500 qPCR Instrument (Software Version 2.4) as an Example:

1) If the threshold needs to be adjusted, set the Threshold of FAM and ROX channel to an appropriate position in the Amplification Plot panel of the Analysis module;

2) In the Plate Setup panel of the Setup module, set the Task of the standard curve wells to "Standard", assign values of 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, 1×10^1 copies/μL in Quantity column, and name them ST6, ST5, ST4, ST3, ST2, ST1 in Sample column. Set the Task of the NTC well to "NTC", set the Task of NCS wells, test sample wells and sample ERC wells to "Unknown", and name them NTC, NCS, S, ERC in the corresponding Sample Name column, then click "Analyze";

3) View slope, intercept, R^2, and amplification efficiency in "Standard Curve".

4) Read concentration (copies/μL) in Quantity column of View Well Table panel of Analysis. Calculate spiking recovery based on test sample and Sample ERC results; recovery shall be 50%–150%.

Precautions

1. Store the kit at -20℃ or below.
2. Read the manual carefully before use; follow the protocol strictly for optimal performance.
3. Use DNase/RNase-free consumables; process quickly and store frozen if not used immediately.
4. Do not use expired components or mix components from different batches.
5. Thaw reagents completely at room temperature and centrifuge briefly before use; avoid repeated freeze-thaw.
6. Use new disposable PE gloves for sealing; avoid bare-hand contact. Use powder-free, non-fluorescent latex gloves.
7. Follow strict PCR laboratory zoning: Reagent Prep → Extraction → Amplification → Analysis. Separate personnel, equipment, reagents, and airflow.
8. Cap samples and calibrators promptly after use to prevent contamination and false positives.
9. Do not open amplification tubes post-reaction. Dispose of waste properly outside the PCR laboratory.
10. Strong positive samples may suppress internal control signal due to competition.

Disclaimer

In all cases, the company's liability for this product is limited to the product's value itself.