

Instructions for Use of RCL (VSVG) Gene Copy Number Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- RC001

Introduction

The RCL (VSVG) Gene Copy Number Detection Kit is a kit specially designed for the quantitative detection of RCL gene copy number in the genome of CAR-T cells prepared by using HIV-1 lentiviral vector technology.

This kit is based on fluorescence probe method and uses multiplex PCR to detect the DNA sequence associated with integration or expression function on the transfer plasmid and calculate the copy number of VSVG gene in the sample. This kit is rapid, highly specific and reliable in the test.

Specification

100 Reactions

Main components

Components	Filling volume	Storage conditions
RCL quantitative standard	50 μ L \times 1 vial	-20 $^{\circ}$ C and below
RCL Primer&Probe MIX	550 μ L \times 1 vial	-20 $^{\circ}$ C and below, protected from light
2x qPCR Reaction Buffer	1.1 mL \times 1 vial	-20 $^{\circ}$ C and below, protected from light
DNA diluent	1 mL \times 3 vials	-20 $^{\circ}$ C and below
ROX High*	50 μ L \times 1 vial	-20 $^{\circ}$ C and below, protected from light
ROX Low*	50 μ L \times 1 vial	-20 $^{\circ}$ C and below, protected from light

Table 1: Kit components

* Please select appropriate ROX for corresponding model.

Storage conditions and shelf life

The shelf life is 18 months when stored at -20 $^{\circ}$ C and below.

Applicable models (including but not limited to)

- ◆ ABI PRISM 7500

- ◆ CFX96(Bio-Rad)
- ◆ Linegene 9600plus (Bioer)

When using with different instrument models, please select the appropriate reference stain ROX.

Instruments	ROX reference stain
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High
Applied Biosystems® 7500, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™, Mx3005P™, and Mx4000™	ROX Low
Rotor-Gene™, DNA Engine Opticon™, Opticon™ 2, Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler®, Roche LightCycle®480, Roche LightCycler®Nano, Bio-Rad CFX96, and Illumina Eco™	No ROX

Prepared by the user

- ◆ 1.5 mL low-attachment sterile centrifuge tube
- ◆ qPCR plate or strip tubes

Operation procedures

I. Dilution of RCL quantitative standard and preparation of standard curve

The concentration of RCL quantitative standard is indicated on the tube wall label; please confirm the concentration before dilution. Convert the number of copies using the following equation:

Plasmid copy number (copies/μL) = $6.02 \times 10^{14} \times \text{Plasmid concentration (ng/μL)} / (\text{Plasmid base number} \times 660)$

Result obtained: RCL gene copy number is 2.00×10^7 copies/μL

II. The detailed procedures for 10-fold gradient dilution of RCL quantitative standard are as follows:

1. Remove the RCL quantitative reference and DNA diluent from the package and place on ice to thaw; after thawing completely, shake gently to mix well, and centrifuge instantly.
2. Take 6 clean 1.5-mL centrifuge tubes and label as STD0, STD1, STD2, STD3, STD4 and STD5, respectively.

The dilution of standards is present in the following table:

Standard No.	Dilution volume	Concentration (copies/μL)
STD0	5 μL of standard + 95 μL of DNA dilution	1.00×10^6
STD1	10 μL STD0 + 90 μL DNA diluent	1.00×10^5
STD2	10 μL STD1 + 90 μL DNA diluent	1.00×10^4
STD3	10 μL STD2 + 90 μL DNA diluent	1.00×10^3
STD4	10 μL STD3 + 90 μL DNA diluent	1.00×10^2

STD5	10 μ L STD4 + 90 μ L DNA diluent	1.00 \times 10 ¹
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III. Preparation and addition of qPCR reaction solution

1. Calculate the required number of reaction wells based on the standard curve and the number of samples to be tested. Generally, 3 replicate wells are required for each sample.

Number of reaction wells = (5 concentration gradients on the standard curve + 1 no template control (NTC) + number of test samples) \times 3.

2. Calculate the total volume of qPCR MIX required for this analysis based on the number of reaction wells.

Volume of qPCR MIX = (number of reaction wells + 2) \times 15 μ L (including volume loss of 2 wells).

3. Take out each reagent and place on ice to thaw, gently shake to mix well, and prepare qPCR MIX according to the table below:

Components	Reaction in single-well
2x qPCR Reaction Buffer	10 μ L
RCL Primer&Probe MIX	4.6 μ L
ROX*	0.4 μ L
Total volume	15 μ L

* Please select appropriate ROX for corresponding model. If there is no ROX suitable for the model, please add deionized water (free of nucleic acid and nuclease contamination) of same volume.

The loading to each reaction well is as follows:

Standard curve	15 μ L qPCR MIX + 5 μ L STD1/STD2/STD3/STD4/STD5
No template control (NTC)	15 μ L qPCR MIX + 5 μ L DNA diluent
Test sample	15 μ L qPCR MIX + 5 μ L test sample

The total liquid volume in each well should be 20 μ L after loading. Gently shake the 96-well plate to mix well, perform rapid centrifugation for 10 seconds, and place the plate in the qPCR system.

IV. qPCR program parameter setting

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Create the experimental reaction program and set a two-step reaction program: the PCR reaction program is as follows: pre-denaturation at 95 $^{\circ}$ C for 2 min; 45 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 15 s, with a reaction volume of 20 μ L.
2. Create an experimental reaction plate, click on "Select Fluorophores" and select fluorescent FAM.

In the reaction plate diagram, select the Sample well, pull down in Sample Type to select Unknown, check the fluorescence FAM, Target Name is designated as RCL; input the number of replicates per sample and Sample Name.

In the reaction plate diagram, select the Standard well, pull down in Sample Type to select Standard, check the fluorescence FAM, and Target Name is designated as RCL; input the number of replicates for each dilution gradient and Sample Name. And the Concentration column of STD1, STD2, STD3, STD4 and STD5 is assigned with values of 100000, 10000, 1000, 100 and 10 copies/ μ L, respectively.

3. Click "Start Run" and choose the save path.

V. qPCR result analysis

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Click Quantitation in Data Analysis Window to read the slope, intercept, amplification efficiency (Effect) and R^2 of the standard curve.
2. In the window Quantitation Data, the SQ Mean column reads the RCL test values of the no-template control (NTC) and the test sample in copies/ μ L.
3. For NTC, the result should be N/A, or the Ct value should be greater than the mean Ct value of the lowest concentration on standard curve.
4. Criteria for negative RCL result: There is no Ct value in 3 replicate wells of the sample or no Ct value in 2 of the 3 replicate wells and there is Ct value in 1 replicate well of the sample, and the Ct value of detected well is greater than the Ct value of LLOQ.
5. Criteria for positive RCL result: If the copy number of VSV-G gene detected in 2 or more wells of the sample is not less than 10 copies/reaction, it is determined as positive for VSV-G gene.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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