Instructions for Use of CAR/TCR Gene Copy Number Detection Kit (Multiplex qPCR)

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

Cat. No. HG- CA001

Introduction

The CAR/TCR Gene Copy Number Detection Kit (Multiplex qPCR) is designed for the quantitative detection of CAR gene copy number in the genome of CAR-T/TCR-T cells prepared by using HIV-1 lentiviral vector technology.

This kit adopts the fluorescent probe method and multiplex PCR method to detect the DNA sequence related to integration or expression function on the transfer plasmid and the reference gene (RFG) in human cells, and the CAR gene copy number/cell in the sample can be calculated. The kit is a rapid, specific and reliable device.

Specification

100 Reactions

Kit components

Components	Filling volume	Storage conditions
CAR quantitative standard	50 μL × 1 vial	-18℃ and below
CAR Primer&Probe MIX	550 μL × 1 vial	-18℃ and below, protected from light
2x qPCR Reacetion Buffer	1.1 mL × 1 vial	-18℃ and below, protected from light
DNA diluent	1.0 mL × 3 vials	-18℃ and below
ROX High*	50 μL × 1 vial	-18℃ and below, protected from light
ROX Low*	50 μL × 1 vial	-18℃ 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 -18℃ 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 IIlumina 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 CAR quantitative standard and preparation of standard curve

The concentration of CAR 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) = 6.02×10^{14} × plasmid concentration (ng/µL)/ (plasmid base pairs × 660)

To obtain: The copy numbers of CAR and RFG genes are both 3.00×10^{8} copies/µL

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

1. Take out the CAR quantitative standard and DNA diluent, place them on ice to thaw completely, then gently shake to mix well, and perform instantaneous centrifugation.

2. Take 7 clean 1.5 mL centrifuge tubes and label them as STD0, STD1, STD2, STD3, STD4, STD5 and STD6, respectively.

3. In the STD0 tube, dilute the CAR quantitative standard with DNA diluent by 10 folds as STD0, shake to mix well, and perform rapid centrifugation for 10 seconds.

4. Add 90 µL of DNA diluent to STD1, STD2, STD3, STD4, STD5, and ST6D tubes, respectively.

5. The dilution of standards is present in the following table.

Standard No.	Dilution volume	Concentration (copies/µL)
STD1	10 µL STD0 + 90 µL DNA diluent	3.00×10^6
STD2	10 µL STD1 + 90 µL DNA diluent	3.00×10^5
STD3	10 µL STD2 + 90 µL DNA diluent	3.00×10^4
STD4	10 µL STD3 + 90 µL DNA diluent	3.00×10^3
STD5	10 µL STD4 + 90 µL DNA diluent	3.00×10^2
STD6	10 µL STD5 + 90 µL DNA diluent	3.00×10^1

Table 2.　　Dilution of standards

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 = (6 concentration gradients on the standard curve + 1 no template control (NTC) + number of test samples) × 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) × 15 µL (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 µL
CAR Primer&Probe MIX	4.6 µL
ROX*	0.4 µL
Total volume	15 µL

* 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 µL qPCR MIX +5 µL STD1/STD2/STD3/STD4/STD5/STD6
No template control (NTC)	15 µL qPCR MIX + 5 µL DNA diluent
Test sample	15 µL qPCR MIX + 5 µL DNA extracting solution of test sample

The total liquid volume in each well should be 20 µL 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 an experimental reaction program and set up a two-step reaction program. The PCR reaction program is as follows: Pre-denaturation at 95℃ for 10 minutes, at 95℃ for 15 seconds, and at 60℃ for 60 seconds; repeat for 40 cycles, with the reaction volume of 20 μL.

2. Create an experimental reaction plate, click on "Select Fluorophores" and select fluorescent FAM and Cy5.

Select sample wells in the reaction plate chart, select "Unknown" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name as "CAR"; check fluorescent Cy5 and name the Target Name as "RFG"; then input the number of replicate wells and Sample Name for each sample.

Select standard curve wells in the reaction plate chart, select "Standard" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name as "CAR"; check fluorescent Cy5 and name the Target Name as "RFG"; then input the number of replicate wells and Sample Name for each dilution gradient. Assign values of 3E+06, 3E+05, 3E+04, 3E+03, 3E+02, 3E+01 (in copies/μL), respectively, to the "Concentration" column of STD1, STD2, STD3, STD4, STD5, and STD6.

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" on the data analysis window to read the slope, intercept, amplification effect, and R^2 of the standard curve.

2. In the "Quantitation Data" window, the CAR and RFG detection values (in copies/μL) of NTC and test samples can be read in the "SQ Mean" column.

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. Result calculation: CAR copy number/cell = 2 × CAR/RFG

Disclaimer

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

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com   info@eaglebio.com

EAGLE
BIOSCIENCES

让细胞药物谱写生命新篇章
CELL THERAPY INNOVATION INSPIRED

©2023 Hillgene. All rights reserved.   Ver. 1.2303.

Jiangsu Hillgene Biopharma Co., Ltd.
Address: Building 4, Yuewang Wisdom Valley, 1463 Wuzhong Avenue, Wuzhong District, Suzhou, China
T.400 900 1882   W.www.hillgene.com