CAR/TCR Gene Copy Number Detection Kit

CAR/TCR Gene Copy Number Detection Kit is designed for precise quantification of CAR or TCR gene copy numbers in biological products.

Catalog number: ARG83102

Package: 100 tests

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INTRODUCTION

This kit 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.

PRINCIPLE OF THE ASSAY

CAR/TCR Gene Copy Number Detection Kit utilizes quantitative polymerase chain reaction (qPCR) technology to accurately detect and quantify CAR or TCR gene copy numbers.

CAR/TCR Gene Copy Number Detection Kit includes a set of primers and probes specifically designed to amplify and detect the targeted DNA sequences associated with CAR or TCR genes. By employing qPCR, which combines amplification and detection in real-time using a fluorescent dye, this kit offers high specificity and sensitivity. It is user-friendly and well-suited for laboratory applications.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at -20 °C. Use the kit before expiration date.

Component	Quantity	Storage information
10X CAR Standard	50 µl	-20°C
CAR Primer & probe mix	550µl	-20°C (protect from light)
2x qRCR Reaction Buffer	1.1 ml	-20°C (protect from light)
DNA Dilution buffer	3 x 1 ml/vails	-20°C
ROX (High)	50µl/vails	-20°C (protect from light)
ROX (Low)	50µl/vails	-20°C (protect from light)

MATERIALS REQUIRED BUT NOT PROVIDED

- PCR machine
- Pipettes and pipette tips
- DNase/RNase-Free Water
- PCR tube

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -20°C at all times.
- All reagents must be kept on ice during the entire experiment.
- Once the assay has been started, all steps should be completed without interruption.
- It is highly recommended that the standards and samples be assayed in triplicates.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

- **Standards**: Dilute 10X CAR Standard with DNA Dilution buffer to yield S6. The DNA Dilution buffer serves as zero standard (0 pg/ml), and the rest of the standard 10-fold serial

Dilute CAR DNA standard as according to the table below:

Standard	DNA Conc. (copies)	µl of DNA Dilution buffer	µl of standard
S6	3 X 10^6	90 µl	10 µl (stock)
S5	3 X 10^5	90 µl	10 µl (S6)
S4	3 X 10^4	90 µl	10 µl (S5)
S3	3 X 10^3	90 µl	10 µl(S4)
S2	3 X 10^2	90 µl	10 µl(S3)
S1	3 X 10^1	90 µl	10 µl(S2)
S0	0l	0 µl	100µl

ASSAY PROCEDURE

1 Prepare qPCR mix buffer:

2x qRCR Reaction Buffer	10µl
CAR Primer & probe mix	4.6µl
ROX *	0.4µl
Total	**15µl (1 wells)**

∗ Choose the appropriate ROX (High or Low) to match the PCR machine. If the PCR machine does not require ROX, adjust the volume with DNase/RNase-free water to obtain a final volume of 15µl.

2 Mix 15µl qPCR mix buffer with 5µl diluent standard / sample / blank in PCR tube. The final volume should be 20 µl.

3 Initial denaturation: 95°C, 10 min

4 PCR cycle:

Denaturation: 95°C, 10 sec

Elongation: 60°C, 15 sec, for **40 cycle, 20 µl.**