SHENTEK

CAR/TCR Transgene Copy Number Quantitation Kit

User Guide

Version: A/1
For Research Use Only
Product No.: SK030221CA100
Reagents for 100 Reactions

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

SHENTEK® CAR/TCR Transgene Copy Number Quantitation Kit is designed to quantify the copy number of target genes in human cell products derived from common HIV-1 lentiviral vectors, such as CAR or TCR genes integrated in CAR-T and TCR-T cell genomes.

This kit utilizes multiplex qPCR technique to detect DNA sequences related to the integration or expression function on the transfer plasmid and Single Copy Gene (SCG) in human cells. Average copies of target gene per cell can be calculated in the samples, such as CAR or TCR gene copy numbers. This kit is rapid, specific, reliable, and universal for lentiviral vector applications. The reference standard in this kit is produced by our company.

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
qPCR Reaction Buffer	NNB001	850 μL × 2 tubes	-20℃, protect from light
CAR/TCR Primer&Probe MIX	NNC032	300 μL × 1 tube	
IPC MIX	NNC066	150 μL × 1 tube	
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	-20℃
CAR/TCR Control	NNA022	50 μL × 1 tube	

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Applied instruments, including but not limited to the following

- SHENTEK-96S Real-Time PCR System

- 7500 Real-Time PCR System
- LineGene 9600plus Real-Time PCR System
- CFX96 Real-Time PCR System

■ Required materials not included in the kit

- Nonstick, RNase-free, Low Retention Microfuge Tubes, 1.5mL
- Nonstick, Low Retention Tips: 1000 μL, 100 μL and 10 μL
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps

■ Related equipment

- Bentchtop microcentrifuge
- Vortex mixer
- Microplate shaker
- Micropipettes 1000 μL, 100 μL and 10 μL
- Real-time PCR Syetem

■ Workflow

Serial dilution of control

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Sample preparation

⇩

qPCR reaction mix preparation

⇩

qPCR amplification

⇩

Data analysis

2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, micropipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.

3. Thaw the kit completely at 2-8°C or melt on ice.

■ Dilution of CAR/TCR Control and preparation of standard curve

Please check the concentration on the label of the CAR/TCR Control tube prior to dilution. Conversion of copy numbers according to the formula:

Plasmid copy numbers (copies/μL) = 6.02×10^{14} × Plasmid concentration (ng/μL) / (Number of plasmid bases × 660)

The copy number of CAR/TCR and SCG is 2.33×10^8 copies/μL

Prepare CAR/TCR Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1. Thaw CAR/TCR Control and DDB completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in a microcentrifuge, and repeat for 3 times.
2. Label six nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.
3. Dilute the CAR/TCR Control for 10 times with DDB in the ST0 tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix it thoroughly.
4. Add 90 μL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.
5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for CAR/TCR Control

Serial dilution tube	Dilution	Conc. (copies/μL)	
		CAR/TCR	SCG
ST1	10 μL ST0 + 90 μL DDB	2.33×10^6	2.33×10^6
ST2	10 μL ST1 + 90 μL DDB	2.33×10^5	2.33×10^5
ST3	10 μL ST2 + 90 μL DDB	2.33×10^4	2.33×10^4
ST4	10 μL ST3 + 90 μL DDB	2.33×10^3	2.33×10^3
ST5	10 μL ST4 + 90 μL DDB	2.33×10^2	2.33×10^2

- *The remaining unused DDB need to be stored at 2-8℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it clear.*

■ Sample preparation

➢ Negative Control Sample (NCS) Preparation

Add 100 μL of DDB to a new 1.5 mL microcentrifuge tube, and label as NCS. NCS and samples should be prepared in same way for DNA extraction.

■ qPCR MIX preparation

1. Determine the number of reaction wells based on the standard curve, with the number of test samples and control samples. Generally, triplicates are tested for each sample.

Number of reaction wells = (5 standard points on the standard curve + 1 NTC + 1 NCS + test samples) × 3

2. Prepare qPCR MIX according to the number of reaction wells.

Table 3. qPCR MIX preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	15.9 μL	524.7 μL
CAR/TCR Primer & Probe MIX	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

3. Mix thoroughly and place on ice, aliquot 20 μL/well into 96-well qPCR plate or PCR 8-strip tubes.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reactions MIX according to Table 4, and a 96-well plate layout template is shown in Table 5.

Table 4. qPCR Reaction MIX preparation

Tubes	Standard curver	NTC	NCS	Test sample
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 5. Example of 96-well plate layout

ST5	ST5	ST5							NTC	NTC	NTC	A
ST4	ST4	ST4							NCS	NCS	NCS	B
ST3	ST3	ST3										C
ST2	ST2	ST2										D
ST1	ST1	ST1										E
									S1	S1	S1	F
									S2	S2	S2	G
									S3	S3	S3	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents the assay for a standard curve with 5 concentration gradients (ST1 to ST5), 1 NTC, 1 NCS, 3 test samples (S1 to S3), and 3 replicates for each sample.*
- *The plate layout for sample loading can be adjusted based on the sample quantity. The recommended total amount of sample loaded per well for the test sample is between 5 ng and 30 ng, which can be adjusted according to your preliminary experiments.*

2. Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it on the qPCR instrument.

■ qPCR program setting

Note: The following instructions apply only to the ABI7500 instrument with SDS v1.4. If you use a different instrument or software, refer to the applicable instrument or software documentation.

1. Create a new document, then in the Assay drop-down list, select **Standard Curve (Absolute Quantitation)**.

2. Click **New Detector**, then enter CAR or TCR in the Name field, select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher; Click **New Detector**, then enter SCG in the Name field, select **CY5** in the Reporter Dye drop-down list and select **(none)** in the Quencher; Click **New Detector**, then

enter IPC in the Name field. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.

3. Select **ROX** as the passive reference dye, then Click **Next**.

4. Select the applicable set of wells for the samples, then select the corresponding detector for each well.

5. Select Finish, and then set thermal-cycling conditions:

a. Set the thermal cycling reaction volume to 30 μL.

b. Set the temperature and time as following (Table 6):

Table 6. qPCR running program

Step	Temp.	Time(mm:sec)	Cycles
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing/extension	60℃*	01:00	

*Instrument will read the fluorescence signal during this step.

6. Save the document, then click **Start** to start the qPCR run.

■ Results analysis

1. Select **Set up** tab, then set tasks for each sample type by clicking on the Task Column drop-down list:

   a. NTC: target DNA detector task = NTC

   b. NCS, test samples= Unknown

2. Set up the standard curve as shown in the following table 7:

Table 7. Settings for Standard curve

Tube label	Task	Conc. (copies/μL)	
		Channel-FAM	Channel-CY5
ST1	Standard	2.33×10^6	2.33×10^6
ST2	Standard	2.33×10^5	2.33×10^5
ST3	Standard	2.33×10^4	2.33×10^4
ST4	Standard	2.33×10^3	2.33×10^3
ST5	Standard	2.33×10^2	2.33×10^2

3. Select the **Results** tab, then select Amplification Plot.
4. In the Data drop-down list, select **Delta Rn vs Cycle**.
5. In the Analysis Settings window, enter the following settings:
   a. Select **Manual Ct**.
   b. In the Threshold field, enter 0.02.
   c. Select **Automatic Baseline**.
6. Click the button ▶ in the toolbar, then wait the plate analyzing.
7. Select the **Result** tab**> >Standard curve** tab, then verify the Slope, Intercept and R^2 values.
8. Select the Report tab, then achieve the mean quantity and standard deviation for each sample.
9. Select **File > > Export > > Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.
10. In the Report panel of Results, the 'Mean Quantity' column shows the detection values of NTC, NCS, test sample, in copies/μL.
11. The Ct value of NTC and NCS should be larger than the mean Ct value of the lowest concentration in the standard curve.
12. Analyze the Ct value of IPC. Normally, the mean Ct-IPC value of the sample should be within ±1.0 of the NCS Ct-IPC value. If the mean Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, this indicates that the sample may be inhibitory to the assay.
13. Calculation of results:

CAR or TCR copies/cell = 2 × CAR or TCR/SCG

Note: The parameter settings of the result analysis should be based on the specific model and the software version, and generally can also be automatically interpreted by the instrument.

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Support & Contact

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