CAR/TCR Gene Copy Number (qPCR) Detection Kit

Catalog Number：CT0311A-100

【Introduction to Kit】

CAR/TCR Gene Copy Number (qPCR) Detection Kit uses TaqMan fluorescent probe method, which can quickly and specifically quantify the copy number of CAR or TCR genes in CAR-T or TCR-T cell genomes, as well as single copy genes (SCG) in human cells, and calculate the average copy number of the target gene per cell in the sample. It has the characteristics of strong specificity and high sensitivity.The linear range of the reagent kit is 3×10^{6} copies/μL-3×10^{1} copies/μL.

【Components of the kit】

Table 1　Composition and storage conditions of the kit

Components	Total amount	Storage Conditions
CAR/TCR Primer&Probe MIX	525 μL×1 tube	-20℃ （keep in dark）
CAR/TCR IPC Primer&Probe MIX	280 μL×1 tube	
2×qPCR Reaction MIX	1050 μL×1 tube	-20℃
CAR/TCR Quantitative Reference （3×10^{8} copies/μL）	50 μL×1 tube	
DNA Diluent	1.5mL×3 tubes	

【Specifications】

100 Reactions

【Period of validity】

Valid for 12 months under the prescribed storage conditions

【Self provided consumables, reagents and equipment required for test】

Fluorescence quantitative PCR instrument

1.5mL centrifuge tube with sterile, and low retention

Optical 8-tube strip or 96-well qPCR plate with sterile, DNase/DNAse-free and low retention

1000μL, 100μL, 10μL pipette gun

1000μL, 100μL, 10μL pipette tip with sterile, low retention and filter element

【Applicable models (including but not limited to)】

ABI QuantStudio 5 Real-Time PCR System

ABI 7500 Real-Time PCR System

Bio-Rad CFX Opus96 Real-Time PCR System

【Testing procedures】

- **Dilution of DNA quantitative reference**

Dilute the DNA quantification reference substance (3×10^8 copies/μL) with the DNA diluent solution provided by the reagent kit at a ratio of 10 times. The diluent concentrations are 3×10^7 copies/μL,3×10^6 copies/μL,3×10^5 copies/μL,3×10^4 copies/μL,3×10^3 copies/μL,3×10^2copies/μL, 3×10^1 copies/μL, respectively. The specific steps are as follows:

1. Melt the DNA quantification reference and DNA diluent in the reagent kit on ice or at 2-8 ℃. After complete dissolution, gently shake and mix well, centrifuge for 3-5 seconds.

2. Take clean 1.5 mL centrifuge tubes and label them as ST0, ST1，ST2，ST3，ST4，ST5，ST6。

3. Dilute the DNA quantification reference substance (3×10^8 copies/μL) in an ST0 tube with DNA diluent to 3×10^7copies/μL, gently shake and mix well, and centrifuge instantly. Ensure that the DNA quantification reference material is thoroughly mixed with the DNA diluent solution, and avoid repeated freezing and thawing of the DNA quantification reference material provided by the kit during use.

4. Add 90 μL of DNA diluent solution to ST1, ST2, ST3, ST4, ST5, and ST6 tubes respectively, and then perform gradient dilution. The dilution method is as follows:

Table 2　Dilution of DNA quantitative reference

Dilution tube	Dilution volume	Final concentration (copies/μL)
ST1	10 μL ST0+90 μL DNA diluent	3×10^6
ST2	10 μL ST1+90 μL DNA diluent	3×10^5
ST3	10 μL ST2+90 μL DNA diluent	3×10^4
ST4	10 μL ST3+90 μL DNA diluent	3×10^3
ST5	10 μL ST4+90 μL DNA diluent	3×10^2
ST6	10 μL ST5+90 μL DNA diluent	3×10^1

Note : 1. The concentration points of the standard curve can be selected according to the actual verification results, and should contain at least 5 concentration points.

2. Melted and unused DNA diluent can be stored at 2-8 °C for a short time. If it is not used for a long time, it should be stored at -20 °C.

3. The dilution method can be adjusted according to the experimental needs.

- **Preparation and sample adding of qPCR reaction solution**

1. Calculate the number of holes required for reaction according to the number of standard samples and samples to be tested (generally 3 groups of multiple holes are made):

Number of reaction holes=(references ST1 to ST6+ NTC+sample to be tested)×3

2. Calculate the total amount of qPCR MIX required for this experiment based on the number of reaction wells： qPCR MIX=(number of reaction wells+2 or 3)× 10 μL (2×qPCR Reaction MIX)+(number of reaction wells+2 or 3)×5 μL (CAR/TCR Primer&Probe MIX) (2 or 3 is the operational loss).

3. Dissolve the reagents completely on ice, shake gently and mix well, centrifuge instantly, and prepare qPCR MIX as shown in Table 3.

Table 3 Preparation of qPCR MIX

Components	Single reaction amount
CAR/TCR Primer&Probe MIX	10 μL
2×qPCR Reaction MIX	5 μL
Total Volume	15 μL

4. Put the required reagent on ice to melt, shake slightly and mix well, and add samples as shown in Table 4 (total volume 20 μL).

Table 4 Sample adding examples of each reaction hole

Template	Template amount	qPCR MIX amount
Reference	5 μL of references ST1 to ST6	15 μL
Template-free NTC	5 μL of each DNA diluent	15 μL
Sample to be tested	5 μL of each sample to be tested	15 μL

- **Preparation and sample adding of IPC qPCR reaction solution（Note: If a sample with added standard has already been set up, IPC can be used as an option）**

1. Calculate the number of holes required for reaction according to the number of standard samples and samples to be tested (generally 3 groups of multiple holes are made):

Number of reaction holes=(references ST1 to ST6+ NTC+sample to be tested)×3

2. Calculate the total amount of IPC qPCR MIX required for this experiment based on the number of reaction wells： IPC qPCR MIX=(number of reaction wells+2 or 3)× 10 μL (2×qPCR Reaction MIX)+(number of reaction wells+2 or 3)×5 μL (CAR/TCR IPC Primer&Probe MIX) (2 or 3 is the operational loss).

3. Dissolve the reagents completely on ice, shake gently and mix well, centrifuge instantly, and prepare **IPC** qPCR MIX as shown in Table 5.

Table 5 Preparation of **IPC** qPCR MIX

Components	Single reaction amount
CAR/TCR **IPC** Primer&Probe MIX	10 μL
2×qPCR Reaction MIX	5 μL
Total Volume	15 μL

4. Put the required reagent on ice to melt, shake slightly and mix well, and add samples as shown in Table 6 (total volume 20μL).（**Note**: **NCS** is an extracted DNA diluent）

Table 6 Sample adding examples of each reaction hole

Template	Template amount	**IPC** qPCR MIX amount
NCS	5 μL of NCS	15 μL
Sample to be tested	5 μL of each sample to be tested	15 μL

5. The test can be performed by using optical 8-tube strip or 96-well qPCR plate. Bubbles in the reaction system should be removed and centrifuged to be bottom of the tube.

- **qPCR reaction procedure and parameter setting**

Taking the American ABI 7500 Real Time PCR System with software version 2.06 as an example.

1. Create a blank new program on the Experiment Properties page:

2. Create a blank new program. Experiment type: Standard curve, Chemistry: TaqMan® Reagents, Run mode: Standard.

3. Create a new detection probe and create a new probe and sample in the Define Targets and Samples interface. Use Add New Targets to add new probes and edit the probe names in Target Name, naming them CAR/TCR,SCG and IPC respectively. Select the desired fluorescent and quenching groups in Reporter and Quencher.The fluorescent group of CAR/TCR is selected as FAM, the quenching group is selected as NONE, the fluorescent group of SCG is selected as VIC, the quenching group is selected as NONE, the fluorescent group of IPC is selected as CY5, the quenching group is selected as NONE, and the reference fluorescence is ROX. Use Add New Samples to add new samples and name them in the corresponding Sample name column.（**Note**: When using IPC for sample detection, select FAM, VIC, and CY5 fluorescence channels simultaneously）

4. Set the reaction procedure

Stage1	Pollution to digest	Reps:1	50℃	2min
Stage2	Predenaturation	Reps:1	95℃	30s
Stage3	Cyclic reaction	Reps:45	95℃	5s
			60℃	34s

Stage3 is set to fluorescence collection at 60℃ for 34s.

5. Click the"Start Run"button in the Run interface to perform PCR measurement.

- **qPCR result analysis**

Taking the American ABI 7500 Real Time PCR System with software version 2.06 as an example.

1. In the Plate interface, set the Task column of standard curve as "S", and assign values to the Quantity column as 3×10^6,3×10^5,3×10^4,3×10^3,3×10^2,3×10^1 (copies/μL) , and name them ST1,ST2,ST3,ST4,ST5 and ST6 in the corresponding Sample name column.

2. In the Plate interface, set the Task column of NTC (no template comparison) as "N", set the Task column of samples to be tested as "U", and name the samples in the corresponding Sample name column.

3. Click Analyze, and in the Amplification Plot panel of Results, preliminarily check whether the shape of the amplification curve is normal.

4. In the Standard Curve panel of Results, you can read the Slope, R^2 and amplification efficiency (Eff%) of the standard curve.

5. In the View panel of Results, you can read the detection value of NTC and the samples to be tested in the column of Mean Quantity, copies/μL.

- The parameter setting of the analysis results depends on the specific model and the software version used, and it can also be automatically interpreted by the instrument.
- The test result of NTC should be undetermined or Ct value >35.
- Based on the differences between the actual sample matrix and NTC matrix, priority should be given to the results of sample spiked recovery rate; If the spiked sample is not tested, the CY5 channel Ct value of the sample is consistent with or ±1 of the CY5 channel Ct value of the NCS. If the Ct value of the CY5 channel in the sample is significantly increased compared to the Ct value of the CY5 channel in the negative control, it indicates that the sample may have significant inhibition.
- Calculation formula: CAR/TCR gene copy number/Cell=$\frac{\text{CAR/TCR gene copy number}}{\text{SCG gene copy number}}\times2$

【Precautions】

1. This kit has passed the verification of stability (freeze-thaw and other factors), and does not need to be divided into multiple tubes.

2. The preparation environment of negative reagents and positive reagents should be divided into different areas, and cannot be operated in one area.

3. Pay attention to replacing the tips in time between different sampling steps to avoid cross contamination, and avoid opening the tube cover for a long time.

4. The kit must be used within the validity period. All components in the kit are recommended to be used after melting in a low temperature environment.

5. Only by strictly observing the operating methods of the instructions and using all the reagents matched with this kit can the best detection effect be ensured.

6. The final test result is closely related to the effectiveness of the reagent, the operator's operation method and the test environment.

7. The company is only responsible for the kit itself, and is not responsible for the sample consumption caused by the use of the kit. Please fully consider the possible usage of samples and reserve enough samples before use.

8. This kit is only for in study, not for clinical diagnosis.

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