TRAP Kit User Manual

CAT#:15-00003 **Low temperature transportation, -20 °C storage**	
Human telomerase activity test **(Probe method TRAP)**	**Agent cartridge**
qPCR Pathogen Detection Kit Series	**User Manual V1.0**

Transportation and storage of self-provided reagents

Telomerase is present in 85-90% of tumor tissues, so it is possible to diagnose most tumors early by detecting telomerase activity. Currently, the most commonly used method for detecting telomerase activity is TRAP (Telomeric Repeat Amplification Protocol), which utilizes the ability of telomerase to add different numbers of TTAGGG sequences to the end of substrate DNA and efficiently detects telomerase activity by detecting extension products through PCR. However, the traditional TRAP method, which is an endpoint electrophoresis detection method, has low sensitivity, cannot be quantified, and is also prone to aerosol contamination. To overcome these shortcomings, our company has developed a TRAP kit based on probe qPCR. It has the following features:

a. Ready to use, providing all reagents from cell lysis to qPCR, eliminating the need for self-optimization.
b. Based on the probe method, fluorescence quantitative PCR has higher sensitivity than electrophoresis TRAP.
c. Using probes, the specificity is higher than that of electrophoresis TRAP.
d. Primers and probes are optimized for high sensitivity, with a minimum detection limit of 100 copies/reaction.
e. Positive control is provided, quantitative analysis can be performed based on this control.
f. One-tube operation eliminates the worry of aerosol pollution.
g. It can be used for both quantitative and qualitative purposes. When used for quantitative purposes, the linear range should be at least 5 orders of magnitude.
h. It can be used for culturing cells as well as for solid tissues (including tumor tissues).

i. This product is sufficient for 50 TRAP PCR assays of a 20μL system.

j. This product can only be used for scientific research.

This product is packaged in a small flat box

Number	Number	Specifications	Packaging
TRAP-specific cell lysate	15-00003a	10 mL	10 mL Natural Bottle
2X TRAP dedicated qPCR MasterMix	15-00003b	0.5 mL	0.5 mL natural cover
Fluorescence PCR template dedicated diluent	180701	1.0 mL	1.5 mL green cap
Human TRAP test positive control (1E7 copies/μL)	pc15-00003	50 μL	0.5 mL Yellow Cap
Human telomerase substrate dry powder	YW15-00003DX-TS	50 times	0.5 mL white cover
Human TRAP primer-probe dry powder	YP15-00003DX	50 times	1.5 mL brown tube
Ultrapure water	210806	1 mL	1.5 mL Blue Cap
User manual	15-00003sc	1 copy	Digital Version Available

Note: When using this product for the first time, add 55μL of ultra-pure water to the human telomerase substrate dry powder; add 165μL of ultra-pure water to the human TRAP primer-probe dry powder tube. Vortex and shake for 1 minute to dissolve, then centrifuge briefly and put it on ice for later use. Unused items need to be stored at -20 °C each time.

Low temperature transportation, -20 °C storage, valid period of 24 months.

BCA Protein Concentration Determination Kit, Solid Phase RNase Scavenger

Method of use

Preparation of standard curve samples

Taking the 6 10x dilutions of positive control 1E1-1E6 copies/μL as an example. Due to the very high concentration of the standard, the following dilution operations must be carried out in an independent area to avoid contaminating the sample or other components of this kit.

a. Mark 6 centrifuge tubes, respectively 6, 5, 4, 3, 2, and 1.

b. Add 45μL of fluorescent PCR dedicated template dilution to tubes 1-6.

c. Add 5μL of 1E7 copies/μL of human TRAP test positive control (provided by the kit) to tube 6, shake well for 1 minute to obtain a standard curve sample of 1E6 copies/μL. Place on ice and set aside.

d. Change the gun head and add 5μL of 1E6 copies/μL of positive control (diluted in the previous step) to the No. 5 tube, and shake it fully

1 minute, get 1E5 copies/μL of standard curve sample. Put it on ice and set it aside.

e. Change the gun head and add 5μL of 1E5 copies/μL of positive control (diluted in the previous step) to the No. 4 tube, and shake it fully

1 minute, get 1E4 copies/μL of standard curve sample. Put it on ice and set it aside.

6. Repeat the above procedure until 6 dilutions of standard curve positive samples are obtained. Set aside on ice. **Two: Extraction of telomerase**

Note: The components of telomerase include RNA, which is easily degraded. Therefore, the extraction of telomerase should be done as quickly as possible under low temperature conditions, and it is best to use our company's solid-phase RNase scavenger to pre-clean the test bench and other areas that are prone to RNase contamination.

g. For frozen solid tissue: grind 50-100 mg of tissue frozen at -80 ° C into powder in a mortar filled with liquid nitrogen, transfer to a pre-cooled glass homogenizer, add 200 μL of pre-cooled TRAP-specific cell lysate, gently homogenize 6 times, then ice bath for 30 minutes, vortex and oscillate every 5 minutes, and then directly enter the first

10 steps operation.

Note: To ensure the lysis effect, observe under a microscope to ensure that most cells have been lysed. If the tissue sample is less than 50-100 mg, the amount of TRAP-specific cell lysate can be reduced proportionally. Solid tissue stored at -20 °C loses telomerase activity after 2 months, while solid tissue stored at -80 °C still has telomerase activity after several years.

h. For fresh cultured cells and tissues: Wash 1E6 cells treated with pancreatic enzymes with self-prepared pre-cooled PBS or

50-100 mg of fresh tissue treated with pancreatic enzymes, centrifuge 3000 g at 4 °C for 5 minutes, discard the supernatant, and the cell pellet can be used directly or stored at -80 °C for subsequent use. Add 200 μL of pre-cooled TRAP-specific cell lysate to the cell or tissue pellet, and gently blow the suspended cells or tissue 3 times. For fresh cells: vortex and shake for 10 seconds, then place in an ice bath for 30 minutes, vortex and shake every 5 minutes. For fresh tissues: use glass on ice

Gently homogenize the glass homogenizer and then ice bath for 30 minutes, vortex and oscillate every 5 minutes. If the cells are less than 1E6

Or 50-100 mg, reduce the dosage of TRAP-specific cell lysate proportionally.

i. Centrifuge 14000 g at 4 °C for 20 minutes, collect 160μL supernatant (containing telomerase), leave 40μL uncollected.

10. Take part of the supernatant and use the self-provided BCA protein concentration determination kit to determine the total protein concentration.
11. After measuring the protein concentration of each sample, use TRAP special cell lysate to adjust the protein concentration of each sample to 3μg/μL, then divide the appropriate amount into centrifuge tubes, put the required amount on ice for later use, and store the rest at -80 °C for a long time (can be stored for one year). The sample obtained in this step is called the telomerase test sample.
12. For each sample, two tubes are required for each experiment, one for measuring telomerase activity and one for the test sample

Heat-inactivated negative control. The preparation method is to take a tube of telomerase test sample from each sample, process it at 95 °C for 10 minutes to inactivate telomerase, and put it on ice for later use. Unused samples can be stored at -80 °C for long-term storage (up to one year) for next use.

2: TRAP probe method (20μL system)

13. Determine the telomerase test sample: For comparison purposes, the total amount of protein (or corresponding number of cells) used for each reaction must be the same, otherwise it is difficult to compare between samples.
14. Set up the reaction. If there are N samples and each sample is repeated once (generally three repetitions are recommended, assuming only one repetition is done for convenience), then 2N + 6 reaction tubes need to be prepared. The extra 1 is because each sample requires a corresponding heat-inactivated negative control. The other 1 is used as a negative control for the probe method TRAP, and the last 5 are used for standard curve samples. Set up the probe method TRAP of the 20μL system according to the table below.

Ingredients	N sample quality control	N heat-inactivated negative pairs Care	Probe method TRAP Negative control	Standard curve sample tubes (1-6 tubes)
Human telomerase substrate solution	1 μL each	1 μL each	1 μL	1 μL
Obtained in step 11 N telomerase test samples	1 μL each	-	-	-
What is obtained in step 12 N heat-inactivated negative control samples	-	1 μL each	-	-
TRAP-specific cell lysate	-	-	1 μL	1 μL
Standard curve sample dilution (No. 1-6) obtained in step 6				5 μL each (No. 2 sample to No. 2 tube, 3 Sample number to 3. Tube...)
Human TRAP primer-probe mixture	3μL each	3μL each	3 μL	3 μL
2X TRAP dedicated qPCR MasterMix	10 μL each	10 μL each	10 μL	10 μL
Ultrapure water	5 μL each	5 μL each	5 μL	-

15. After blowing and mixing, telomere extension and PCR amplification were performed on the machine. The reaction parameters are as follows:

Process	Temperature	Time
Telomere elongation	30°C	30 min
Predenaturation	95°C	5 min
PCR reaction	95°C	15 sec

		(45 cycles)	57.5°C	15 sec	
			72°C	30 sec (acquisition of FAM channels, quenching groups to MGB)	
		Note: If using the ABI 7500 qPCR instrument, the renaturation temperature of 57.5 °C needs to be changed to 48 °C.			
	III: **Analysis of Results** p. The validity judgment of the experiment: If the FAM signal result of the standard curve sample tube is negative (no Ct value, or greater than or equal to 35), the entire experiment is invalid, and data analysis is not required. The experiment needs to be repeated or contacted with the manufacturer. If the FAM signal results of the TRAP negative control tube by probe method are all positive (with Ct values less than 35), it indicates environmental pollution, and the entire experiment is invalid. There is no need to analyze the data and contact the manufacturer. If the FAM signal result of the standard curve sample tube is positive and the result of the TRAP negative control tube by probe method is negative, the experiment is valid and can proceed to the next step of analysis. q. Standard curve production: Using the log values of 5 standard curve sample concentrations as the horizontal axis and the Ct values of the positive control (FAM channel) as the vertical axis, draw the standard curve. The standard curve of the positive control is a diagonal line, and r2 must be greater than 0.95. Then, calculate the log value of the copy number of the telomere repeat sequence synthesized by the telomerase from the standard curve of the positive control using the Ct value of the telomere sample to be tested, and then calculate the copy number of the newly synthesized telomere repeat sequence based on this log value. Since the copy number of the newly synthesized telomere DNA repeat sequence is related to the activity of telomerase. Therefore, the size of telomerase activity is inversely correlated with the tested Ct, and the Ct values obtained from the same experiment can be used to compare the measured Ct values The relative size of telomere activity in this study.				
Related products	Telomere length detection kit				