NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Telomerase Assay Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1802950
Reagents for 200 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Telomerase Assay Kit (Real-time Quantitative TRAP method, or RQ-TRAP method) follows a telomeric repeat amplification protocol (TRAP) that is designed as a dual fluorescence qPCR system. The dual functional assay has employed an internal reference gene to exclude the possibility of false negatives caused by potential inhibitors in the samples. Meanwhile, TSR8 is selected as a reference material for accurate quantification of telomerase activity in test samples, providing a highly sensitive and accurate quantification for telomerase activity. In addition, this kit is a closed-cap assay without tedious operations such as gel electrophoresis or ELISA analysis, which greatly improves the efficiency of the assay and also reduces the risk of cross contamination.

■ 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

No.	Reagent	Part No.	Quantity	Storage
I	Cell Lysis Buffer	NND058	1.5 mL × 5 tubes	-20℃
	RNase inhibitor	NND060	50 μL × 1 tube	
	Telomerase positive cells Pellet	NNA060	10^5 Cells × 2 tubes	
II	2 × TRAP qPCR Reaction Buffer	NNB022	650 μL × 4 tubes	
	TRAP qPCR Reaction Enzyme	NNC108	100 μL × 2 tubes	
	TSR8	NNA059	50 μL × 1 tube	
	TRAP Primer&Control MIX	NNC109	300 μL × 2 tubes	-20℃, protect from light
	TRAP qPCR Reaction Replenisher	NND059	650 μL × 2 tubes	

Note: According to USP <659>, items with recommended storage temperatures not exceeding –20°C shall be stored within ± 10°C (–30°C to –10°C). Short-term

storage below –30°C is acceptable when supported by stability data.

The kit components can be stored at appropriate conditions for up to 18 months. Please check the expiration date on the labels.

■ Applied instruments, including but not limited to the following

- ABI 7500 Real-Time PCR System
- SHENTEK-96S Real-Time PCR System
- LightCycler 480II Real-Time PCR System

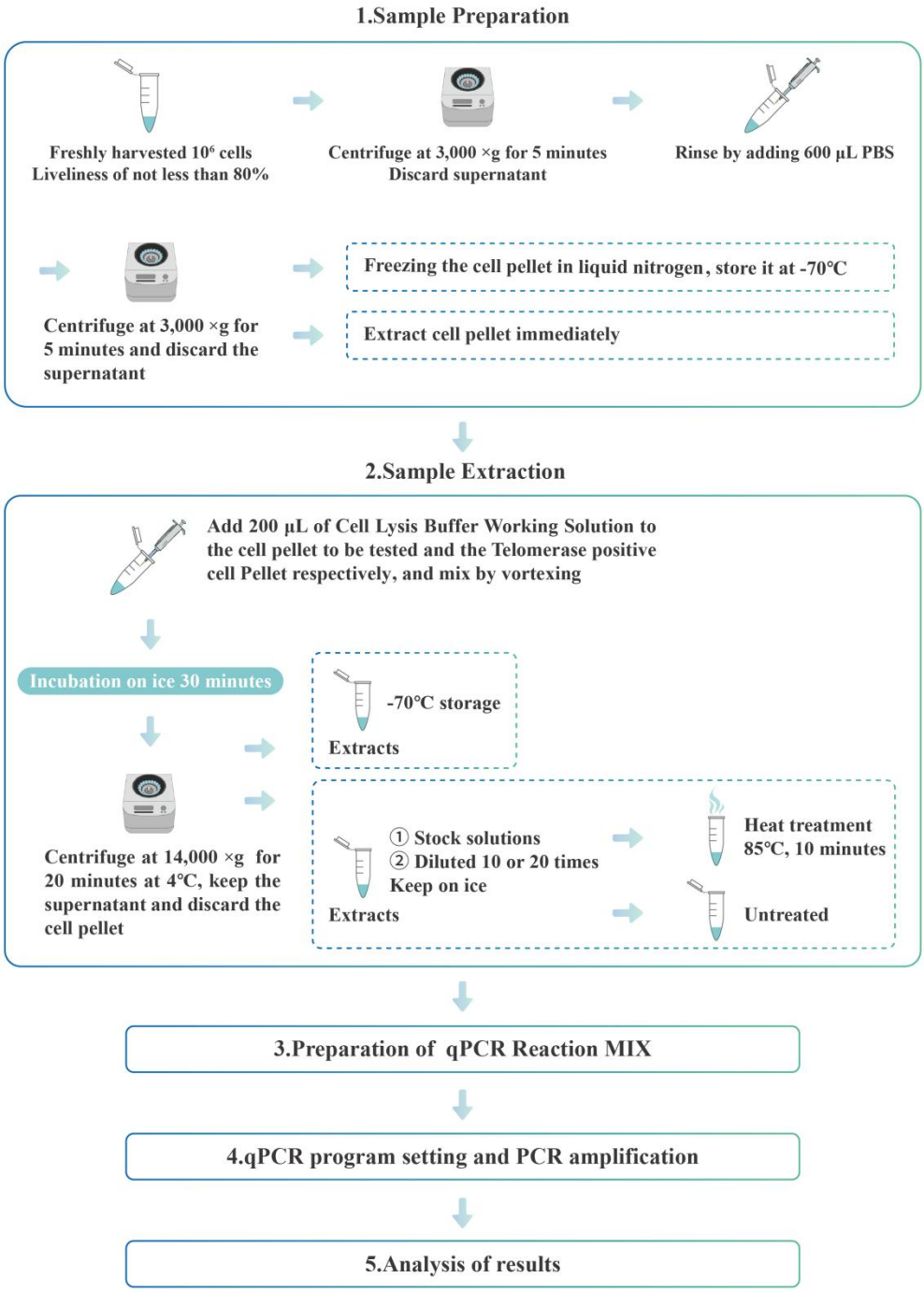
■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes
- Low retention, sterile filter tips
- 96-well qPCR plates with seals or PCR 8-strip tubes with caps
- PBS without Magnesium and Calcium (pH 7.4)

■ Related equipments

- Real-Time PCR System
- Vortex mixer
- Benchtop microcentrifuge
- Pipettes: 1000 μL, 200 μL, 100 μL and 10 μL
- Dry bath incubator or water bath
- High-speed refrigerated centrifuge

■ Workflow



Laboratory Setup and Precautions

One of the most important considerations for this Telomerase Assay Kit is the environment control, where the initial reaction mixtures are prepared. To prevent false-negative and false-positive results, the ideal environment should be free of contaminants listed below.

(1) TSR8 control template

(2) Ribonucleases

(3) Amplified PCR DNA products

Some sources of TSR8 control template and PCR product contamination are:

(1) contaminated pipettes and tips

(2) tube racks

(3) notebooks

(4) lab coats

(5) any other item exposed to amplified PCR products.

Some sources of RNase contamination are:

(1) solutions and tubes not treated with an RNase inhibitor;

(2) any equipment handled without gloves.

The following precautions should be followed in all steps of the assay protocol including sample preparation & extraction and the Telomerase Assay Kit assay setup.

(1) Physically separated areas are recommended for Telomerase Assay procedures: sample preparation area, positive area, negative area, and PCR amplification area.

(2) When pipetting TSR8 control template, always use a designated set of pipettes exclusively, and make sure not to use these pipettes in any other steps of the assay. Wear gloves and use aerosol resistant tips (RNase free).

(3) Keep the assay solutions (TSR8 and Telomerase positive cells Pellet) separate from other reagents in the lab.

(4) Post amplification TRAP procedures should never be carried out near the Telomerase Assay preparation areas.

(5) Clean the PCR tube racks with 10% bleach and UV irradiation after each use.

Note: *The four areas can be located in separate rooms. If space is limited, the sample preparation area, positive area, and negative area may be divided within the same room, but PCR amplification area must be in a separate room. In addition, pipettes, tips, tubes, and other equipment from different areas should be strictly separated and used independently to avoid cross-contamination.*

2. Methods

■ Sample Preparation

(1) Preparation of Cell Lysis Buffer Working Solution (Handle in Negative Area)

a. Calculate the amount of Cell Lysis Buffer Working Solution used for the experiment.

Total volume = 400 μL (standard curve preparation) + 200 μL × Number of samples (sample extraction) + 50 μL × Number of samples (sample dilution, optional)

b. Preparation of Cell Lysis Buffer Working Solution

The working solution is prepared in the negative area by adding 5 μL RNase inhibitor (NND060) to 1 mL Cell Lysis Buffer (NND058), and keep on ice for further use.

(2) Sample Preparation (Handle in Sample Preparation Area)

a. Take freshly harvested 10^6 cells (cell viability not less than 80% required), centrifuged at 3,000 ×g for 5 minutes, and discard the supernatant carefully, then rinse the cells with 600 μL PBS.

b. Centrifuge at 3,000 ×g for 5 minutes, carefully discard the supernatant and retain the cell pellet.

The following operations can be performed depending on the actual situation:

Case 1: If the sample extraction cannot be performed immediately, the cell pellets can be stored at -70℃ and below for up to one year without repeated freezing and thawing.

Case 2: If the sample extraction can be performed immediately, the cell pellets can be placed on ice for further use.

Note: *In this step, the supernatant should be removed as much as possible after centrifugation, only the cell pellets should be retained.*

■ Sample Extraction (Handle in Sample Preparation Area)

(1) Sample extraction

a. Centrifuge the lyophilized Telomerase positive cells Pellet (NNA060) at 14,000 g

for 1 minute at 4℃.

b. Add 200 μLof Cell Lysis Buffer Working Solution to the cell pellet to be tested a nd the Telomerase positive cells Pellet respectively, mix briefly by vortexing, and centrifuge quickly for 3 seconds.

Note: *Cell suspension should be homogenized after addition of Cell Lysis Buffer Working Solution. If cell clusters are visible, vortex until the cells are homogeneous.*

c. Incubate the cells on ice (or at 2-8℃) for 30 minutes, centrifuge at 14,000 ×g for 20 minutes at 4℃, transfer 160 μL of the supernatant to a fresh tube as sample extract and place on ice.

Note: *Use centrifuge tubes certified for high-speed centrifugation to ensure safe operation.*

We recommend that the remaining extract be aliquoted in minimal portions and stored at -70℃ to avoid repeated freezing and thawing, and the storage time should not exceed 6 months.

(2) Treatment of sample extracts

a. Dilutions (If required)

Take 5 μL of sample extract and perform a 10-fold dilution, i.e. add it to 45 μL of Cell Lysis Buffer Working Solution, mix it and place on ice.

Note: *Since PCR inhibitors may be present in the samples, the following suggestions will help to decide whether the sample extracts need to be diluted:*

① *For samples with high telomerase activity (e.g., tumor cells, spontaneously immortalized cells and hiPSCs, etc.), a 10-fold dilution for the pilot study or direct extraction of 10^5 cells is recommended;*

② *For samples with low telomerase activity (e.g., primary cells, limited cell lines, hMSCs, etc.), it is recommended to detect the sample extracts directly for the pilot study.*

③ *For samples with unknown telomerase activity, at least two concentrations of sample extracts are suggested for the pilot study, as for the original sample extracts and a 10-fold dilution of the sample extracts;*

Note: *The above suggestions are for reference only and the optimal dilution of the sample extracts will be based on your actual experience. Sample extracts should always be placed on ice to maintain telomerase activity. Extracts from Telomerase positive cells Pellet can be tested directly without dilution.*

b. Heat treatment

Transfer 10 µL of the original or diluted sample extract to a new 1.5 mL microcentrifuge tube, incubate at 85°C for 10 minutes in a dry bath or water bath, and slowly cool it down to room temperature, then quickly spin for 3 seconds to collect the solutions.

Note: *The heat-treated sample extract is designed to inactivate telomerase, and serves as a negative control in the assay. Heat treatment is required for all sample extracts.*

■ Preparation of qPCR Reaction MIX

(1) Preparation of qPCR MIX (Handle in Negative Area)

a. Thaw all reagents on ice or at 2-8°C, mix thoroughly and prepare qPCR MIX according to the table below.

Table 2. qPCR MIX Preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
2 × TRAP qPCR Reaction Buffer	12.5 µL	412.5 µL
TRAP qPCR Reaction Enzyme	1 µL	33 µL
TRAP Primer&Control MIX	3 µL	99 µL
TRAP qPCR Reaction Replenisher	6.5 µL	214.5 µL
Total	23 µL	759 µL

b. Thoroughly mix the qPCR MIX and aliquot 23 µL/well into a 96-well qPCR plate or PCR 8-strip tubes. Place on ice until use.

(2) Preparation of standard curve (Handle in Positive Area)

a. Dilute the TSR8 (NNA059) to 200 amol/µL in a clean 1.5 mL microcentrifuge tube with Cell Lysis Buffer Working Solution according to the labeled

concentration and label as ST0.

b. Label six clean 1.5 mL microcentrifuge tubes as ST1, ST2, ST3, ST4, ST5 and ST6.

c. Perform 10-fold serial dilution from ST0 tube to ST6 with Cell Lysis Buffer Working Solution, as described in the following table.

Table 3. Dilution for TSR8

Serial dilution tube	Dilution	Conc. (amol/μL)	TPG Units
ST0	Dilute the TSR8 with Cell Lysis Buffer Working Solution	200	400000
ST1	5 μL ST0 + 45 μL Cell Lysis Buffer Working Solution	20	40000
ST2	5 μL ST1 + 45 μL Cell Lysis Buffer Working Solution	2	4000
ST3	5 μL ST2 + 45 μL Cell Lysis Buffer Working Solution	0.2	400
ST4	5 μL ST3 + 45 μL Cell Lysis Buffer Working Solution	0.02	40
ST5	5 μL ST4 + 45 μL Cell Lysis Buffer Working Solution	0.002	4
ST6	5 μL ST5 + 45 μL Cell Lysis Buffer Working Solution	0.0002	0.4

Note:

- *The prepared reference standards can be stored at 2-8℃ for the same day use only, and the reference standards should be mixed well before use.*
- *To ensure the accuracy of the dilution, the minimum sampling size for TSR8 should be not less than 5 μL.*
- *Since the standard is ssDNA, mix gently and avoid excessive shaking.*
- *At least five concentrations of standard curve should be included. To select appropriate sample dilutions, we recommend to perform method validation before sample testing.*
- *1 amol TSR8 = 1000 TPG Units*

(3) Preparation of qPCR Reaction MIX

a. Prepare qPCR Reaction MIX in a 96-well qPCR plate or PCR 8-strip tubes, sample extracts, TSR8 (quantitative reference) tube of ST1 - ST6 and others, as shown in the table below:

Table 4. qPCR Reaction MIX preparation

Operation Area	**Negative Area**	**Sample Preparation Area**			**Positive Area**
Tubes	**No template control**	**Negative quality control**	**Test sample**	**Positive quality control**	**Standard curve**
qPCR MIX	23 μL	23 μL	23 μL	23 μL	23 μL
Templates	2 μL Cell Lysis Buffer Working Solution	2 μL Heat Treatment Sample Extracts	2 μL Sample Extracts	2 μL Telomerase positive cells Extracts	2 μL ST1 - ST6
Total Volume	25 μL	25 μL	25 μL	25 μL	25 μL

Note:

- *When adding samples, pay attention to the recommended areas for each sample type. The recommended order for adding samples is: negative area, sample preparation area and positive area.*
- *Mix sample extracts and ST1 - ST6 tubes again before use.*
- *Due to the small pipetting volume of the template and the viscosity of the Cell Lysis Buffer Working Solution, rinse the pipette tips by pipetting up and down several times when adding the template to ensure adequate amount is added.*

Table 5. Example of 96-well plate layout

NTC	NTC	NTC	PC	PC	PC				ST6	ST6	ST6	A
HT-S1	HT-S1	HT-S1	S1	S1	S1				ST5	ST5	ST5	B
HT-S2	HT-S2	HT-S2	S2	S2	S2				ST4	ST4	ST4	C
HT-S3	HT-S3	HT-S3	S3	S3	S3				ST3	ST3	ST3	D
HT-S4	HT-S4	HT-S4	S4	S4	S4				ST2	ST2	ST2	E
HT-S5	HT-S5	HT-S5	S5	S5	S5				ST1	ST1	ST1	F
HT-S6	HT-S6	HT-S6	S6	S6	S6							G
HT-S7	HT-S7	HT-S7	S7	S7	S7							H
1	2	3	4	5	6	7	8	9	10	11	12	

Note:

- *This example includes selected standard curve points of TSR8 Control (ST1-ST6), NTC (No template control), HTS1-S7 (Negative quality control), Test Samples (S1-S7) and PC (Positive quality control), with 3 replicates for each sample.*

- *The plate layout for sample loading can be adjusted based on the sample quantity.*

b. Seal the 96-well plate with an optical membrane or the 8-strip tubes with caps accordingly, vortex briefly to mix well and centrifuge for 10 seconds. Place it on the qPCR instrument and setup the qPCR programme as follows.

■ qPCR Program Setting (Handle in PCR Amplification Area)

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)**. Click **New Detector**, then enter **Target** in the Name field, select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**. Click **New Detector**, then enter **IC** in the Name field. Select **CY5** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.

(2) Select **ROX** as the passive reference dye, then Click **Next**.

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

(4) Select Finish, and then set thermal-cycling conditions:

a. Set the thermal cycling reaction volume to 25 μL.

b. Set the temperature and time as following:

Table 6. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	30℃	30:00	1
Activation	95℃	02:00	1
Denaturation	95℃	00:15	35
Annealing	50℃	01:00	
Extension*	68℃	00:30	

*Instrument will read the fluorescence signal during this step.

(5) Save the document, then click **Start** to start the qPCR run.

(6) Edit the plate information following the steps:

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

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

Table 7. Settings for Standard curve

Tube label	Task	Quantity (TPG Units)
ST1	Standard	40000
ST2	Standard	4000
ST3	Standard	400
ST4	Standard	40
ST5	Standard	4
ST6	Standard	0.4

c. Select the **Results** tab, then select Amplification Plot.

d. In the Data drop-down list, select **Delta Rn vs Cycle**.

e. In the Analysis Settings window, enter the following settings :

a. Select **Manual Ct**.

b. In the Threshold field, enter 0.1.

c. Select **Automatic Baseline**.

f. Click the button  in the toolbar, then wait the plate analyzing.

g. Select the **Result** tab**> >Standard curve** tab, then verify the Slope, Intercept and R^2 values .

h. Select the Report tab, then achieve the mean quantity and standard deviation for each sample.

i. Select **File > > Export > > Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.

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.*

■ Results Analysis

(1) Criteria for Standard curves: amplification efficiency within the range of 83.3%-110%, $R^2 \geq 0.990$.

(2) Criteria for No template control (NTC) and Negative quality control (Heat Treatment Sample):

a. Reference gene (CY5): The mean Ct values are within 1 cycle difference from the mean Ct values of the standard curve and NTC.

b. Target gene (FAM): The Ct values are greater than the detection limit.

If the above conditions are satisfied, the negative control is considered as qualified; If the Ct value of the target gene is less than or equal to the detection limit, indicates that there is contamination in the detection system, and further investigation is required.

(3) Criteria for Positive quality control (Telomerase positive cells Pellet):

a. Reference gene (CY5): The mean Ct values are within 1 cycle difference from the mean Ct values of the standard curve and NTC.

b. Target gene (FAM): Positive signal detected.

If the above conditions are satisfied, the Positive quality control is considered as qualified.

(4) For Test samples (when no template control, negative QC and positive QC all meet the experimental requirements):

a. Reference gene (CY5): If the Ct value difference between the mean Ct value of standard curve and NTC is less than 1 cycle, consider that the test sample does not inhibit the reaction. If the Ct value is more than 1 cycle, indicates that the test sample may be inhibitory on the reaction, and suggested to repeat the test with optimized dilution of the sample.

b. Target gene (FAM):

a) Calculation of the number of cells per reaction for the test sample:

$$\frac{\text{Total number of cells(A)}}{\text{200 μL (Cell Lysis Buffer Working Solution)} \times \text{Dilution factors (B,if available)}} \times \text{2 μL (Template volume/reaction)}$$

$$= \text{cells/reaction}$$

For example:

If total number of HEK293 cells is 10^6 (A) ,and dilution factors is 10-fold (B), then the number of cells per reaction of the test sample is 1000 cells, count as 1000 cells/reaction.

$$\frac{1{,}000{,}000 \text{ cells}}{200\ \mu L \times 10} \times 2\ \mu L = 1000 \text{ cells/reaction}$$

b) The detection value (TPG Units) of the target gene (FAM) in each assay well was obtained by the software, and the telomerase activity could be calculated as the number of cells per reaction for test sample.

For example: 1000 cells/reaction of HEK293 cells target gene detection value is 300 TPG Units, which means every 1000 HEK293 cells equals to the telomerase activity is 300 TPG Units.

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Support & Contact

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