Telomerase Activity Quantification qPCR Assay Kit (TAQ)

Catalog #8928
100 reactions

Product Description

Telomeres are repetitive nucleotide elements at the ends of chromosomes that protect chromosomes from degradation and genetic information loss. Telomerase is an RNA-containing enzyme that synthesizes the telomere repeat sequence at the 3' end of telomeres. Normal diploid cells lack telomerase activity; therefore, telomeres are lost with each cell cycle. In certain cells such as embryonic stem cells and most cancer cells, however, telomerase is activated to maintain telomere length and cellular immortality. The quantitative detection of telomerase activity is important in many aspects of cell biology, such as stemness and oncogenesis.

ScienCell's Telomerase Activity Quantification qPCR Assay Kit (TAQ) is designed to quantitatively compare telomerase activity among cell populations. The cell lysis buffer is a mild lysis buffer that enables the release of telomerase in the native state. The telomere primer set (TPS) recognizes and amplifies newly synthesized telomere sequences in the assay. The carefully designed primers ensure at suggested assay conditions: (i) high efficiency for trustworthy quantification; and (ii) no non-specific amplification. The primer set has been validated by qPCR with melt curve analysis and gel electrophoresis for amplification specificity and by template serial dilution for amplification efficiency. The 2X GoldNStart TaqGreen qPCR Master Mix (Cat #MB6018a-1) is a SYBR®Green dye-based qPCR master mix with a "hot-start" property. It contains SYBR®Green, dNTPs, Taq DNA polymerase, and an inert gold-color loading indicator in a single tube. The "hot-start" property achieved through ScienCell's unique chemically modified Taq DNA polymerase provides maximal inhibition of primer dimer formation. The advanced buffer formulation provides superior specificity and efficiency with a wide linear dynamic range. The inert gold-color loading indicator allows for better visualization and tracking of sample loading in qPCR plates or tubes.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2X GoldNStart TaqGreen qPCR master mix	1 mL	-20°C
8928a	Cell lysis buffer	10 mL	-20°C
8928b	5X Telomerase reaction buffer	400 µL	-20°C
8928c	Telomere primer set (TPS), lyophilized	1 vial	-20°C
8928d	Nuclease-free H_2O	4 mL	4°C
8928e	Telomerase-positive cell lysate	11 µL	-80°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Cell pellet	Customers' samples
0.1 M PMSF	0.1 M PMSF stock solution in isopropanol, stored at -20 °C
β-mercaptoethanol	
qPCR plate/tube and seal	

Quality Control

TAQ is validated by various telomerase positive and negative human and mouse cells. The specificity of the primer sets is validated by qPCR with melt curve analysis, the PCR products are analyzed by gel electrophoresis, and the efficiency of the primer sets are validated by template serial dilution (See **Appendix**).

Product Use

TAQ is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product TAQ is shipped on dry ice. Upon receipt, store the telomerase-positive cell lysate (Cat #8928e) at -80°C, store the GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) in the dark at -20°C in a manual defrost freezer, store the buffers (Cat #8928a and 8928b) and the telomere primer set (Cat #8928c) at -20°C in a manual defrost freezer, and store the nuclease-free H_2O (Cat #8928d) at 4°C. Once thawed, do NOT refreeze GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1), and keep in the dark at 4°C or on ice at all times.

Procedures

Important: We recommend ***only*** *using polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only*** *use nuclease-free reagents in PCR amplification.*

The following procedure takes approximately 8 hours with three stop points available as indicated in the procedures below. To pause during experimental procedure, stop ONLY at the designated stop points. When comparing the telomerase activity among samples, the samples must be processed in parallel throughout the entire procedure.

A. Preparation of cell lysate

1. For each sample, count cell numbers to be harvested. Harvesting 2-5 million cells/sample is recommended. Wash cells with PBS once, pellet cells and carefully remove PBS. (Stop point #1: cell pellets can be stored at -80 °C freezer).

2. Maintain the cell pellets, cell lysates and reagents at 4 °C or on ice unless otherwise stated through the procedure.

3. Directly before use, thaw 0.1 M PMSF in isopropanol (not provided) at 37 °C until crystals disappear and mix well.

4. Determine the total volume of cell lysis buffer (Cat #8928a) to be used for the samples at 20 µL/million cells (see an "example of calculations" below). Transfer the calculated amount of cell lysis buffer with 5% extra to a new pre-chilled tube. Supplement the aliquoted cell lysis buffer with 0.1M PMSF and β-mercaptoethanol (not provided). For every milliliter of cell lysis buffer, add 1µL 0.1M of PMSF and 0.3 µL of β-mercaptoethanol. Store PMSF at -20 °C after use immediately.

5. Transfer the exact amount of supplemented cell lysis buffer to each cell pellet sample at 20 µL/million cells. Carefully pipette the cell pellet up and down 20 times with a 1 mL pipette tip without generating bubbles. The samples should be homogenous. If not, continue pipetting until fully homogenized. Leave the homogenized samples at 4 °C for 30 minutes.

6. Spin the samples at 12,000-16,000x g for 20 minutes at 4 °C. Carefully transfer 15 µL of supernatant/million cells to a new pre-chilled tube without disturbing the pellet. (Stop point #2: cell lysates can be flash-frozen with dry ice and stored at -80 °C freezer for up to 1 month).

Example of calculations: Sample A has 2.6 million cells and sample B has 4.7 million cells.

In step **A4**, aliquot (2.6 + 4.7) x 20 µL x 105% = 153 µL of cell lysis buffer (Cat #8928a), then add 153 µL x 1 µL/1 mL = 0.153 µL of 0.1 M PMSF and 153 µL x 0.3 µL/1 mL = 0.046 µL of β-mercaptoethanol to the aliquoted cell lysis buffer. Alternatively, first dilute 0.1 M PMSF 1:5 and dilute β-mercaptoethanol 1:20 by adding 1 µL of 0.1 M PMSF to 4 µL of cell lysis buffer (Cat #8928a) and 1 µL of β-mercaptoethanol to 19 µL of cell lysis buffer (Cat #8928a), then add 0.153 µL x 5 = 0.77 µL of diluted PMSF and 0.046 µL x 20 = 0.92 µL of diluted β-mercaptoethanol to the aliquoted 153 µL of cell lysis buffer (Cat #8928a).

In step **A5**, transfer 2.6 x 20 µL = 52 µL of supplemented cell lysis buffer to sample A, and 4.7 x 20 µL = 94 µL of supplemented cell lysis buffer to sample B.

B. Telomerase reaction setup

1. When using this kit for the first time, prepare a telomerase-negative cell lysate sample by transferring 5.5 µL of telomerase-positive cell lysate (Cat #8928e) to a new tube and heating it at 85 °C for 10 min to inactivate it. Briefly spin down the tube at 1,500x g for 10 seconds and leave on ice.

2. Thaw 5X telomerase reaction buffer (Cat #8928b) and leave on ice.

3. Prepare telomerase reactions for each sample as shown in Table 1. Include a positive control using 0.5 µL of telomerase-positive cell lysate sample (Cat #8928e), and a negative control using 0.5 µL of telomerase-negative cell lysate sample. The kit provides enough reagents for 10 positive controls and 10 negative controls. Store telomerase-positive and -negative samples at -80 °C after use immediately.

   Recommended: Set up another "no-lysate" negative control reaction by adding 0.5 µL of supplemented lysis buffer (no cell lysate added).

   Table 1.

Cell lysate sample or cell lysis buffer	0.5 µL
5X Telomerase reaction buffer (Cat #8928b)	4 µl
Nuclease-free H_2O (Cat #8928d)	15.5 µl
Total volume	***20 µl***

4. Incubate the reactions at 37 °C for 3 hours.

5. Stop the reactions by heating the samples at 85 °C for 10 minutes. Briefly spin down the reaction tubes at 1,500x g for 10 seconds and leave on ice. (Stop point #3: post reaction samples can be stored at -20 °C freezer for up to 3 days, or at -80 °C freezer for up to 1 month. Repeated freeze-and-thaw cycles should be avoided).

C. qPCR setup

1. When using this kit for the first time, allow the telomere primer set (TPS, lyophilized, Cat #8928c) vial to warm to room temperature.

2. Centrifuge the vials at 1,500x g for 1 minute.

3. Add 200 µL nuclease-free H_2O (Cat #8928d) to telomere primer set (TPS, lyophilized, Cat #8928c) to make TPS stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

4. Prepare qPCR reactions with TPS stock solution for the post telomerase reaction samples. For maximum reliability, replicates are highly recommended (minimum of 3). Prepare 20 µl qPCR reactions as shown in Table 2.

Recommended: Set up another "no-template" negative control qPCR reaction by adding H_2O (no post telomerase reaction sample added).

Table 2.

Post telomerase reaction sample or H_2O	1 µl
Primer stock solution (TPS)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8928d)	7 µl
Total volume	***20 µl***

5. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.

6. Refer to Table 3 for qPCR program setup. The 2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) contains SYBR®Green as the reporter dye and does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.

   Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of telomere primer set (TPS, Cat #8928c), we highly recommend an annealing temperature of 52°C as shown in Table 3:

Table 3.

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	36
Annealing	52°C	20 sec	
Extension	72°C	45 sec	
Data acquisition	Plate read		
Optional	*Melting curve analysis*		1
Hold	20°C	Indefinite	1

D. Results interpretation and Calculations: Comparative Cq Method

Note: Please refer to your qPCR instrument's data analysis software for data analysis. The method provided here serves as guidance for quick manual calculations.

1. $Cq_{(TPS)}$ is the Quantification Cycle Value obtained from the qPCR program using the Telomere primer set (TPS). Any sample with a $Cq_{(TPS)}$ value higher than 33 is considered having undetectable telomerase activity. In this case do not proceed to the calculations below.

Table 4. Interpretation of $Cq_{(TPS)}$ value.

Sample $Cq_{(TPS)}$ value	Interpretation
> 33	telomerase activity undetectable
< 33	positive telomerase activity

2. $\Delta Cq_{(TPS)}$ is the quantification cycle value difference between the two samples.

   $\Delta Cq_{(TPS)} = Cq_{(TPS,\ sample\ 2)} - Cq_{(TPS,\ sample\ 1)}$

   Note: the value of $\Delta Cq_{(TPS)}$ can be positive, 0, or negative.

3. Relative telomerase activity of sample 2 to sample 1 (fold) = $2^{-\Delta Cq}$

Example Calculations:

Table 5. $Cq_{(TPS)}$ values of two samples.

	Sample 1	***Sample 2***
$Cq_{(TPS)}$	31.68	27.32

$\Delta Cq_{(TPS)} = Cq_{(TPS,\ sample\ 2)} - Cq_{(TPS,\ sample\ 1)}$

$= 27.32 - 31.68$

$= -4.36$

Relative telomerase activity of sample 2 to sample 1 (fold) = $2^{-\Delta Cq}$

$= 2^{4.36}$

$= 20.5$

Conclusions: The telomerase activity of sample 2 is 20.5-fold higher than that of sample 1.

Appendix: Quality assessment of telomere primer set (TPS)

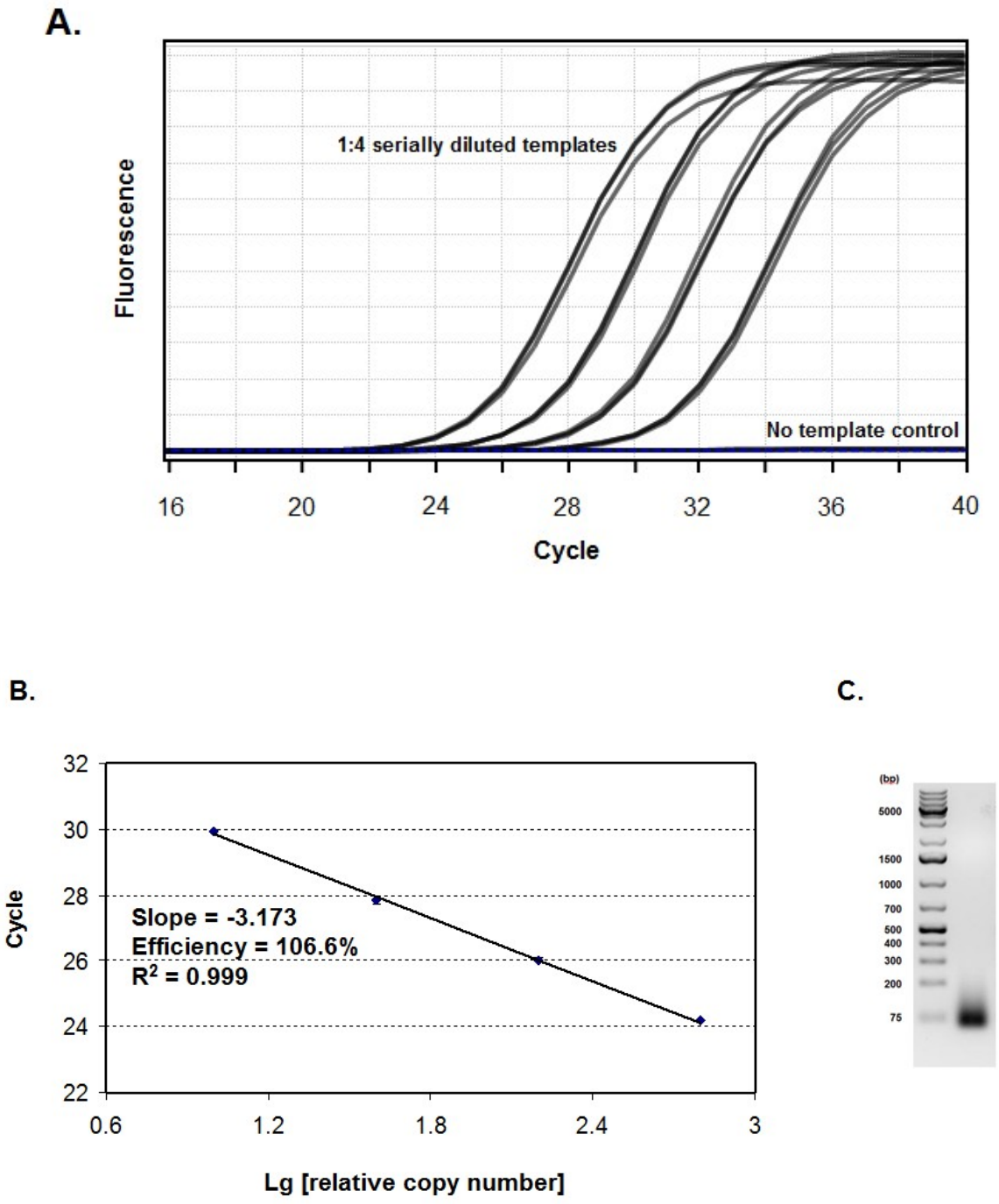

Figure 3. Quality assessment of the telomere primer set (TPS). (A) qPCR amplification curves using serially diluted templates. **(B)** Derivation of qPCR efficiency of TPS. **(C)** Separation of qPCR product by gel electrophoresis. A smeared band is observed as expected.