Rev. 0

Human TERT DNA Copy Number Quantification qPCR Assay Kit (HTCQ)

Catalog #9018
100 reactions

Product Description

Telomerase reverse transcriptase (TERT) is a vital enzyme that maintains telomere length, crucial for cellular immortality and genome stability. The human TERT gene (hTERT) copy number often varies in cancer cells, serving as a key marker in oncogenesis studies. Increased hTERT copy number is linked to cancer development, tumor progression, poor prognosis, and genomic instability by enhancing telomerase activity. During cell immortalization, hTERT integration into the genome is common, making hTERT copy number measurement essential for distinguishing normal cells from immortalized ones.

ScienCell's Human TERT Copy Number Quantification qPCR Assay Kit (HTCQ) delivers a robust and accurate method to quantify hTERT copy number, driving insights into cancer development. The kit detects hTERT copy number in cell lines immortalized via hTERT overexpression and validates their consistency. The hTERT primer set amplifies a 100 bp-long region of the human TERT gene on chromosome 5. The single copy reference (SCR) primer set targets a 100 bp-long region on human chromosome 17. The reference DNA sample consists of a 1:1 ratio of hTERT copies to SCR copies and serves as a reference for calculating the ratio of hTERT to SCR of target samples.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2X GoldNStart TaqGreen qPCR master mix, 1 mL	2 vials	-20°C
9018a	hTERT primer set, lyophilized	1 vial	-20°C
9018b	Human single copy reference (SCR) primer set, lyophilized	1 vial	-20°C
9018c	Nuclease-free H_2O	4 mL	4°C
9018d	Reference DNA sample (hTERT: SCR = 1:1)	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Product Name
DNA isolation kit	SpeeDNA Isolation Kit (ScienCell, Cat #MB6918)
genomic DNA template	Customers' samples
qPCR plate or tube	

Quality Control
The hTERT and SCR primer sets are validated for specificity by qPCR with melt curve analysis and gel electrophoresis. Amplification efficiency is confirmed by template serial dilution (see Appendices 1 and 2).

Product Use
HTCQ 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 is shipped on dry ice. Upon receipt, store the GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) in the dark at -20°C in a manual defrost freezer, the primers (Cat #9018a and 9018b) and the reference DNA sample (Cat #9018d) at -20°C in a manual defrost freezer, and the nuclease-free H_2O (Cat #9018c) 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: ***Only*** *use polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only*** *use nuclease-free reagents in PCR amplification.*

1. Prior to use, allow vials (Cat #9018a and #9018b) 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 #9018c) to hTERT primer set (lyophilized, Cat #9018a) to make hTERT primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
4. Add 200 µl nuclease-free H2O (Cat #9018c) to SCR primer set (lyophilized, Cat #9018b) to make SCR primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
5. For the reference DNA sample (Cat #9018d), prepare two qPCR reactions, one with hTERT primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 1.

Table 1.

Reference DNA sample	2 µl
Primer stock solution (hTERT or SCR)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #9018c)	6 µl
Total volume	***20 µl***

6. For each genomic DNA sample, prepare two qPCR reactions, one with hTERT primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 2.

Table 2.

Genomic DNA template (0.5 – 5 ng/µl)	2 µl
Primer stock solution (hTERT or SCR)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #9018c)	6 µl
Total volume	***20 µl***

7. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds. For maximum reliability, replicates are highly recommended (minimum of 3).

8. 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 hTERT and SCR primer sets (Cat #9018a and #9018b), we highly recommend an annealing temperature of 62°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	40
Annealing	62°C	20 sec	
Extension	72°C	45 sec	
Data acquisition	Plate read		
Optional	*Melting curve analysis*		1
Hold	20°C	Indefinite	1

Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) 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. For hTERT, ΔCq (hTERT) is the quantification cycle number difference of hTERT between the target and the reference DNA samples.

   ΔCq (hTERT) = Cq (hTERT, target sample) - Cq (hTERT, reference sample)

   Note: the value of ΔCq (hTERT) can be positive, 0, or negative.

2. For single copy reference (SCR), ΔCq (SCR) is the quantification cycle number difference of SCR between the target and the reference DNA samples.

   ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)

   Note: the value of ΔCq (SCR) can be positive, 0, or negative.

3. $\Delta\Delta Cq = \Delta Cq$ (hTERT) - ΔCq (SCR)
4. The ratio of hTERT to SCR of the target sample

   $= 2^{-\Delta\Delta Cq}$

5. The hTERT copy number of the target sample

   $= 2 \times 2^{-\Delta\Delta Cq}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 3. Cq (Quantification Cycle) values of hTERT qPCR (hTERT) and single copy reference qPCR (SCR) obtained for the genomic DNA samples.

Primer set	*Target sample*	*Reference sample*
hTERT	24.83	20.99
SCR	25.64	20.80

ΔCq (hTERT) = Cq (hTERT, target sample) - Cq (hTERT, reference sample)
= 24.83 - 20.99
= 3.84

ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)
= 25.64 - 20.80
= 4.84

$\Delta\Delta Cq = \Delta Cq$ (hTERT) - ΔCq (SCR)
= 3.84 - 4.84
= -1

The ratio of hTERT to SCR of the target sample
$= 2^{-\Delta\Delta Cq}$
$= 2^{-(-1)}$
$= 2$

Conclusions: *The average hTERT copy number in the target genomic DNA sample is twice that of the SCR control. Assuming there are 2 copies of SCR per diploid cell, this suggests there are 4 copies of hTERT per cell (2 × 2 = 4).*

Appendix 1: Quality assessment of hTERT primer set

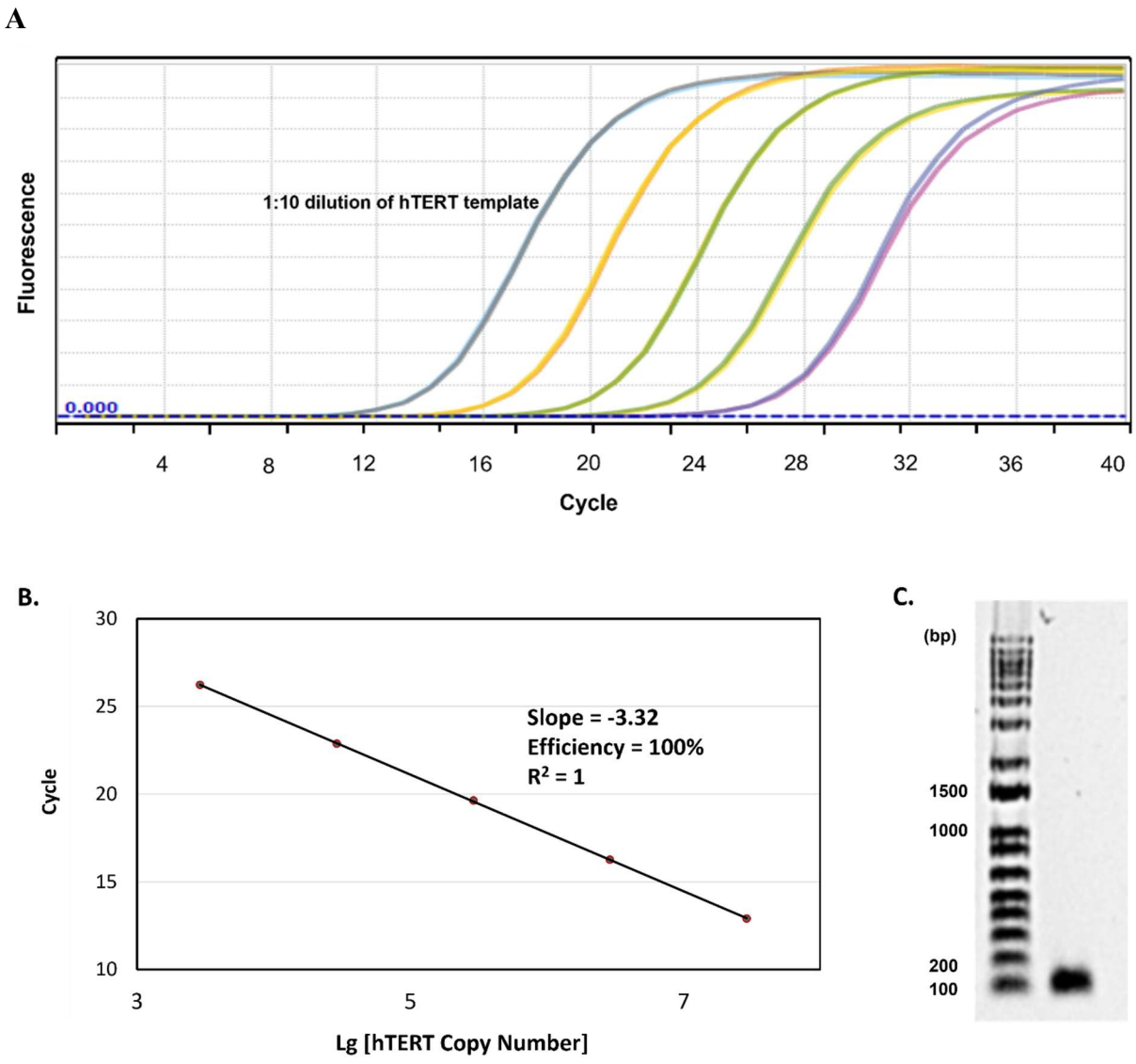

Figure 1. Quality assessment of hTERT primer set. (A) qPCR amplification curves using serially diluted hTERT template. **(B)** Derivation of qPCR efficiency of hTERT primer set. **(C)** Separation of hTERT qPCR product by gel electrophoresis.

Appendix 2: Quality assessment of Single copy reference (SCR) primer set

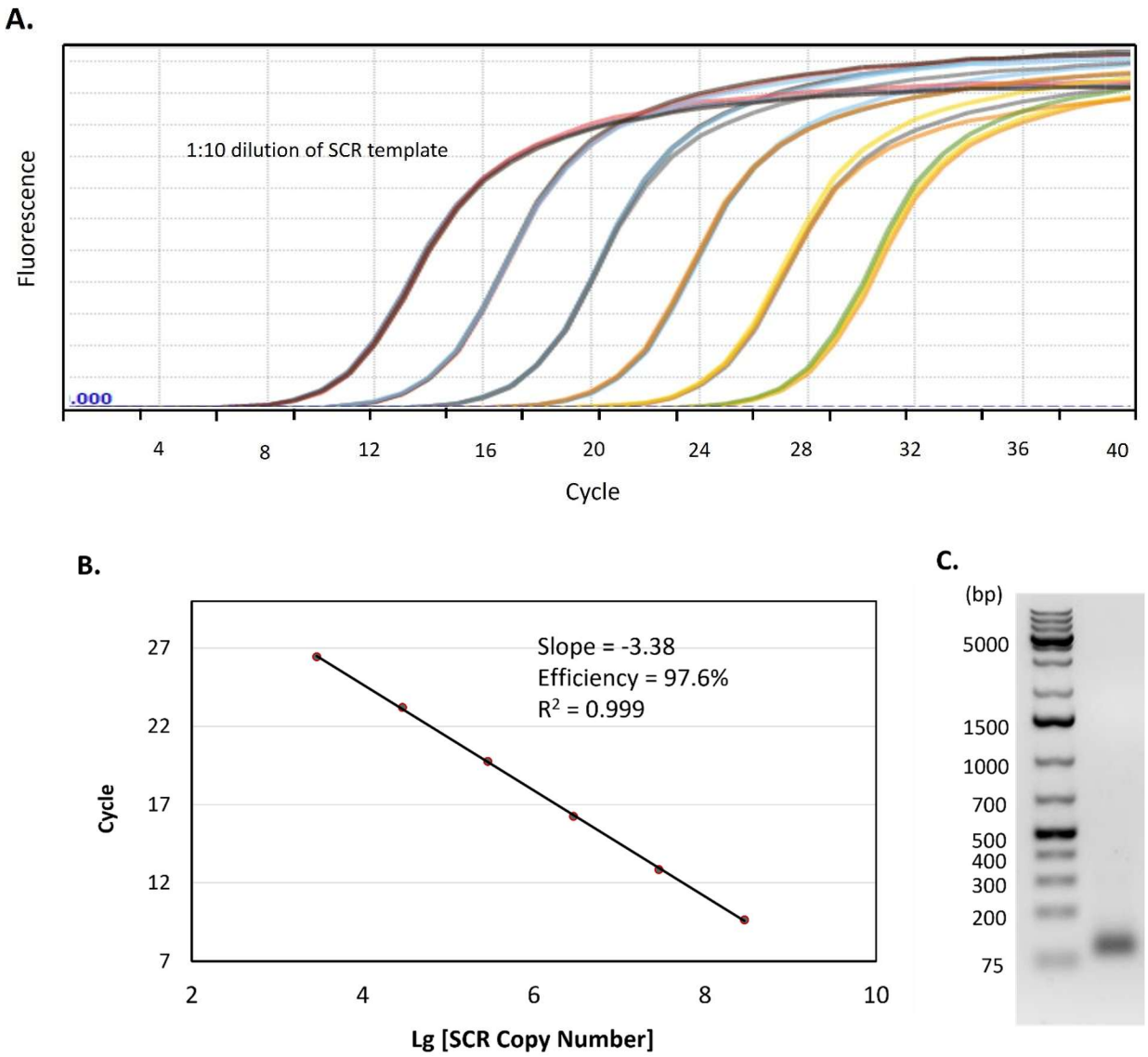

Figure 2. Quality assessment of Single copy reference (SCR) primer set. (A) qPCR amplification curves using serially diluted SCR template. **(B)** Derivation of qPCR efficiency of SCR primer set. **(C)** Separation of SCR qPCR product by gel electrophoresis.