Rev.1

Absolute Human Telomere Length Quantification qPCR Assay Kit (AHTLQ)

Catalog #8918
100 reactions

Product Description

Telomeres are repetitive nucleotide elements at the ends of chromosomes that protect chromosomes from degradation and genetic information loss. Normal diploid cells lose telomeres with each cell cycle. Telomere length, therefore, decreases over time and may predict lifespan. Telomere shortening has negative effects on health conditions and has been linked to many health issues including aging and cancer. Accurate and consistent quantification of telomere length is important in many aspects of cell biology such as chromosomal instability, DNA repair, senescence, apoptosis, cell dysfunctions, and oncogenesis.

ScienCell's Absolute Human Telomere Length Quantification qPCR Assay Kit (AHTLQ) is designed to directly measure the average telomere length of a human cell population. The telomere primer set recognizes and amplifies telomere sequences. The single copy reference (SCR) primer set recognizes and amplifies a 100 bp-long region on human chromosome 17, and serves as reference for data normalization. The reference genomic DNA sample with known telomere length serves as a reference for calculating the telomere length of target samples. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) no non-specific amplification. Each 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	2 vials	-20°C
8918a	Telomere primer set, lyophilized	1 vial	-20°C
8918b	Single copy reference (SCR) primer set, lyophilized	1 vial	-20°C
8918c	Nuclease-free H_2O	4 mL	4°C
8918d	Reference Human genomic DNA sample (Lot #37789, Lot #35318, telomere length: 1.23 ± 0.09 Mb per diploid cell)	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
genomic DNA template	Customers' samples
qPCR plate or tube	

Note: Components Cat. #8918a and Cat. #8918b are lyophilized and a pellet may not be visible in the vials.

Quality Control

The specificity of the primer sets is validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis. The efficiency of the primer sets is validated by template serial dilution (See **Appendices 1 and 2**). The telomere length of reference genomic DNA sample is quantified against a reference gDNA with known telomere length.

Product Use

AHTLQ 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 #8918a and 8918b) and the reference genomic DNA sample (Cat #8918d) at -20°C in a manual defrost freezer, and the nuclease-free H_2O (Cat #8918c) 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.

Procedure:

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 #8918a and #8918b) 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 #8918c) to telomere primer set (lyophilized, Cat #8918a) to make telomere 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 #8918c) to SCR primer set (lyophilized, Cat #8918b) 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 genomic DNA sample (Cat #8918d), prepare two qPCR reactions, one with telomere 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 genomic DNA sample	1 µl
Primer stock solution (Telomere or SCR)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8918c)	7 µl
Total volume	***20 µl***

6. For each genomic DNA sample, prepare two qPCR reactions, one with telomere 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)	1 µl
Primer stock solution (Telomere or SCR)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8918c)	7 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 telomere and SCR primer sets (Cat #8918a and #8918b), 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	32
Annealing	52°C	20 sec	
Extension	72°C	45 sec	
Data acquisition	Plate read		
Optional	*Melting curve analysis*		1
Hold	20°C	Indefinite	1

Figure 1. A typical amplification curve showing the amplification of a qPCR product.

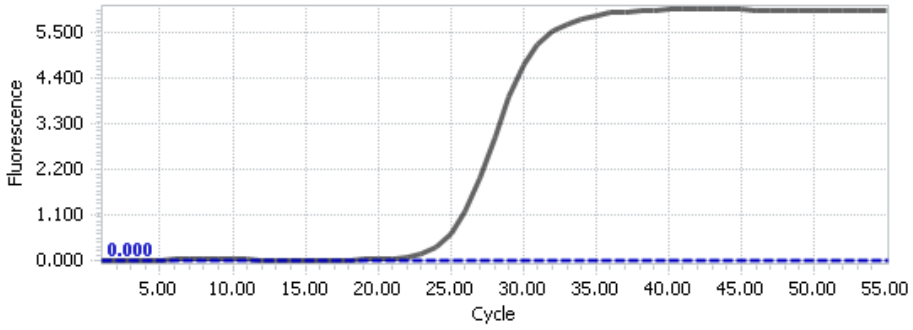
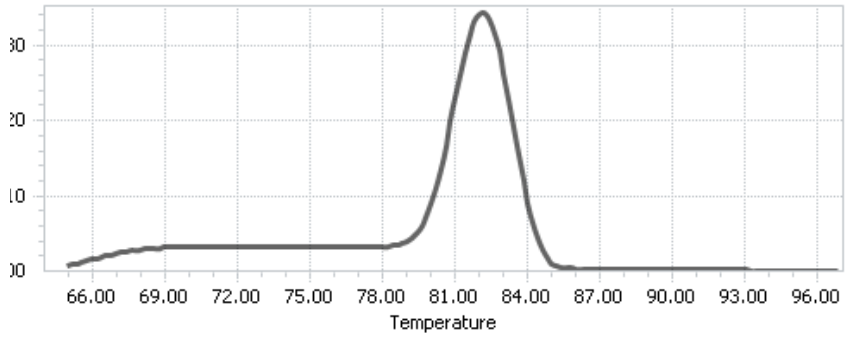


Figure 2. A typical melting peak of a qPCR product.



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 telomere (TEL), ΔCq (TEL) is the quantification cycle number difference of TEL between the target and the reference genomic DNA samples.

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

   Note: the value of ΔCq (TEL) 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 genomic 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. ΔΔCq = ΔCq (TEL) - ΔCq (SCR)

4. Relative telomere length of the target sample to the reference sample (fold) = $2^{-\Delta\Delta Cq}$

5. The total telomere length of the target sample

   = Reference sample telomere length x $2^{-\Delta\Delta Cq}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

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

Primer set	*Target sample*	*Reference sample*
TEL	13.12	14.18
SCR	24.64	26.10

ΔCq (TEL) = Cq (TEL, target sample) - Cq (TEL, reference sample)
= 13.12 – 14.18
= -1.06

ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)
= 24.64 - 26.10
= -1.46

$\Delta\Delta Cq = \Delta Cq\ (TEL) - \Delta Cq\ (SCR)$
$= -1.06- (-1.46)$
$= 0.40$

Relative telomere length of the target sample to the reference sample (fold)
$= 2^{-\Delta\Delta Cq}$
$= 2^{-0.40}$
$= 0.76$

The total telomere length of the target sample per diploid cell
= Reference sample telomere length x $2^{-\Delta\Delta Cq}$
$= (1.23 \pm 0.09\ Mb) \times 0.76$
$= 935 \pm 68\ kb$

There are 92 chromosome ends in one diploid cell, therefore, average telomere length on each chromosome end = $(935 \pm 68\ kb) / 92$
$= 10.2 \pm 0.7\ kb$

Conclusions: The average telomere length of target genomic DNA sample is 935 ± 68 kb per diploid cell, or 10.2 ± 0.7 kb per chromosome end.

Appendix 1: Quality assessment of Telomere primer set

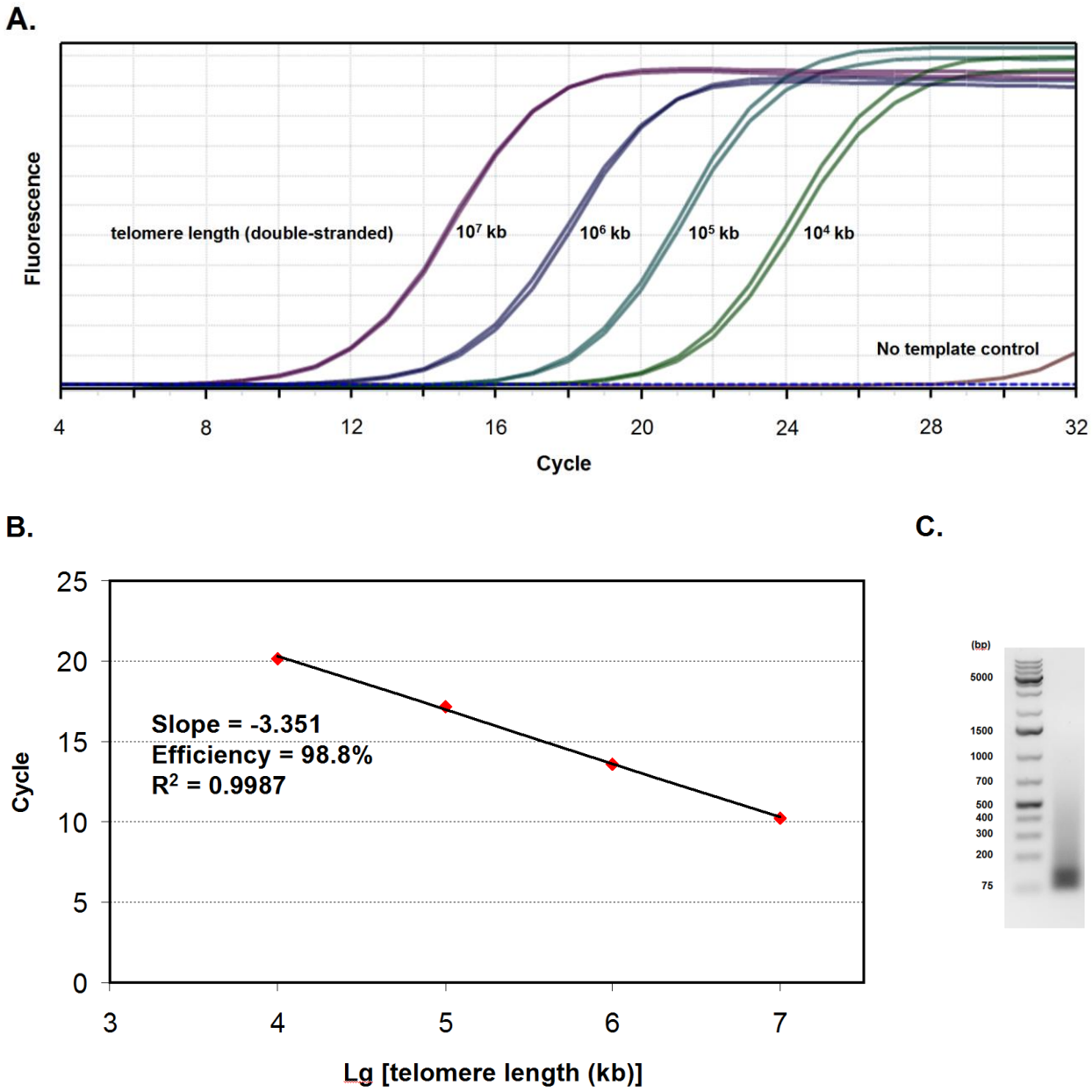


Figure 3. Quality assessment of Telomere primer set. (A) qPCR amplification curves using serially diluted telomere repeats as template. **(B)** Derivation of qPCR efficiency of Telomere primer set. **(C)** Separation of Telomere qPCR product by gel electrophoresis. A smeared band is observed as expected.

Note: Due to the tandemly repeated nature of telomere sequences, Telomere primer set may exhibit trace amounts of primer dimer formation in No Template Control (NTC) reactions. A Cq value of 28 or greater indicates the primer dimer formation in a NTC reaction and can be treated as a negative result. If the Cq value for a telomere reaction using a test sample is less than 20, then the formation of primer dimers will NOT affect the quantification of telomere length.

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

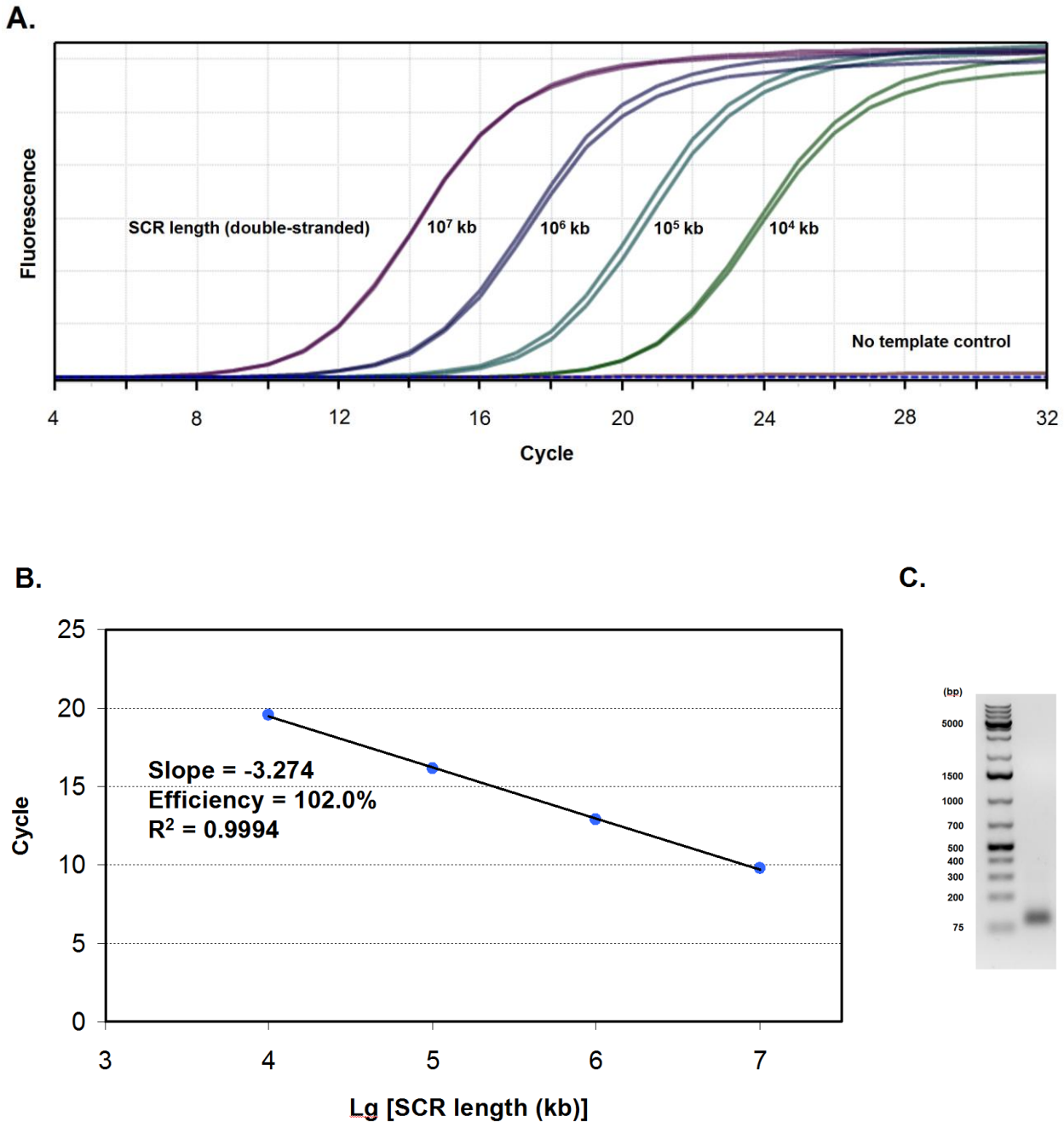


Figure 4. Quality assessment of Single copy reference (SCR) primer set. (A) qPCR amplification curves using serially diluted SCR template. **(B)** Derivation of qPCR efficiency of SCR primer set. **(C)** Separation of SCR qPCR product by gel electrophoresis.