Absolute Human Mitochondrial DNA Copy Number Quantification qPCR Assay Kit (AHMQ)

Catalog #8948
100 reactions

Product Description

Mitochondrial DNA (mtDNA) is circular, multicopy genome DNA located in mitochondrion, a cellular organelle that plays a key role in the energy production. The capacity for energy production in a cell depends on both mtDNA integrity and copy number. Substantial evidence suggests that alterations in mtDNA copy number has been correlated with aging and various age-related disorders, such as cancer, diabetes and neurodegenerative diseases.

ScienCell's Absolute Human Mitochondrial DNA Copy Number Quantification qPCR Assay Kit (AHMQ) is designed to quantify the average mtDNA copy number of a human cell population. The mtDNA primer set recognizes and amplifies one of the most conserved regions on human mtDNA and will not amplify any off-target sequence on nuclear genomic DNA. 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 mtDNA copy number serves as a reference for calculating the mtDNA copy number 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.

Kit Components

Cat #	Component	Qty.	Storage
8948a	Human mtDNA primer set, lyophilized	1 vial	-20°C
8948b	Human single copy reference (SCR) primer set, lyophilized	1 vial	-20°C
8948c	Nuclease-free H_2O	4 mL	4°C
8948d	Reference Human genomic DNA sample (Lot #26172, mtDNA copy number: $(1.20 \pm 0.04) \times 10^3$ copies per diploid cell)	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
DNA isolation kit	DNeasy Blood & Tissue Kit (Qiagen, Cat #69504, 69506)
genomic DNA template	Customers' samples
qPCR plate or tube	
qPCR master mix	FastStart Essential DNA Green Master (Roche, Cat #06402712001)

Quality Control

The specificity of the primer sets are validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis. The efficiency of the primer sets are validated by template serial dilution (See **Appendices 1 and 2**). The mtDNA copy number of reference genomic DNA sample is determined by qPCR standard curve method (See **Appendix 3**).

Product Use

AHMQ 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 primers (Cat #8948a and 8948b) and the reference genomic DNA sample (Cat #8948d) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #8948c) at 4°C.

Procedures

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

Note: The quality of the qPCR master mix is a critical element for successful qPCR analyses. AHMQ is optimized using FastStart Essential DNA Green Master (Roche, Cat #06402712001) and is highly recommended. Use of other qPCR master mixes may compromise results.

1. Prior to use, allow vials (Cat #8948a and #8948b) 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 #8948c) to mtDNA primer set (lyophilized, Cat #8948a) to make mtDNA 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 #8948c) to SCR primer set (lyophilized, Cat #8948b) 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 #8948d), prepare two qPCR reactions, one with mtDNA 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 (mtDNA or SCR)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #8948c)	7 µl
Total volume	***20 µl***

6. For each genomic DNA sample, prepare two qPCR reactions, one with mtDNA 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
Primer stock solution (mtDNA or SCR)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #8948c)	variable
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 strongly recommended (minimum of 3).

8. For qPCR program setup, refer to Table 3 when using FastStart Essential DNA Green Master (Roche, Cat #06402712001). This master mix 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. When using other qPCR master mixes, the qPCR program may require optimization with Table 3 as a starting protocol.

Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of mtDNA and SCR primer sets (Cat #8948a and #8948b), 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	
Annealing	52°C	20 sec	32
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.

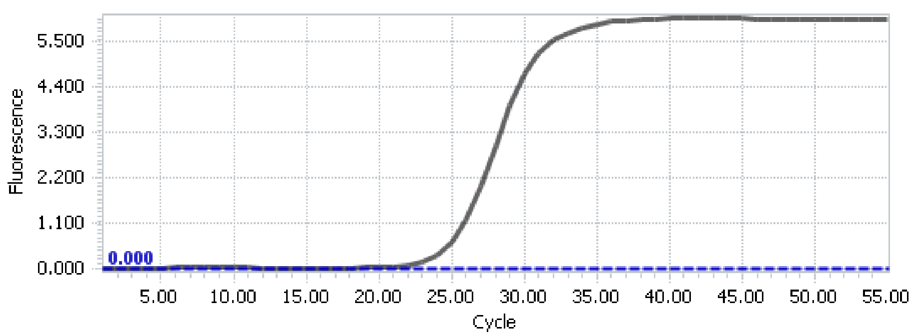
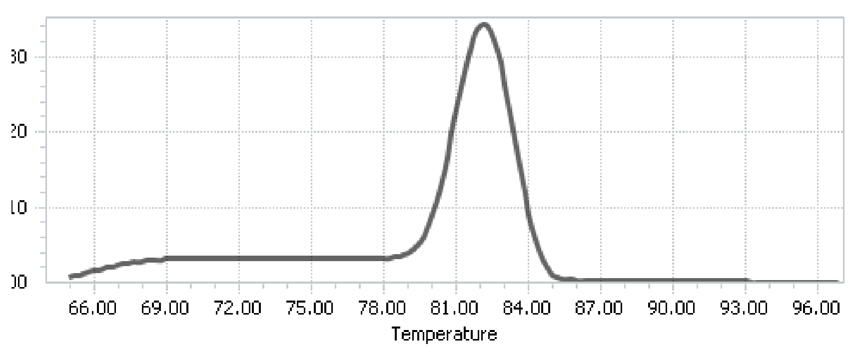


Figure 2. A typical melting peak of a qPCR product.



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

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

   Note: the value of ΔCq (mtDNA) 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 (mtDNA) - ΔCq (SCR)

4. Relative mtDNA copy number of the target sample to the reference sample (fold)

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

5. The mtDNA copy number of the target sample

   = Reference sample mtDNA copy number x $2^{-\Delta\Delta Cq}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

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

Primer set	*Target sample*	*Reference sample*
mtDNA	14.62	16.68
SCR	24.64	26.10

ΔCq (mtDNA) = Cq (mtDNA, target sample) - Cq (mtDNA, reference sample)
= 14.62 - 16.68
= -2.06

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

$\Delta\Delta Cq = \Delta Cq$ (mtDNA) - ΔCq (SCR)

$= -2.06 - (-1.46)$

$= -0.60$

Relative mtDNA copy number of the target sample to the reference sample (fold)

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

$= 2^{0.60}$

$= 1.52$

The mtDNA copy number of the target sample per diploid cell

= Reference sample mtDNA copy number x $2^{-\Delta\Delta Cq}$

$= (1.20 \pm 0.04) \times 10^3 \times 1.52$

$= (1.82 \pm 0.06) \times 10^3$

Conclusions: The average mtDNA copy number of target genomic DNA sample is $(1.82 \pm 0.06) \times 10^3$ per diploid cell.

Appendix 1: Quality assessment of mtDNA primer set

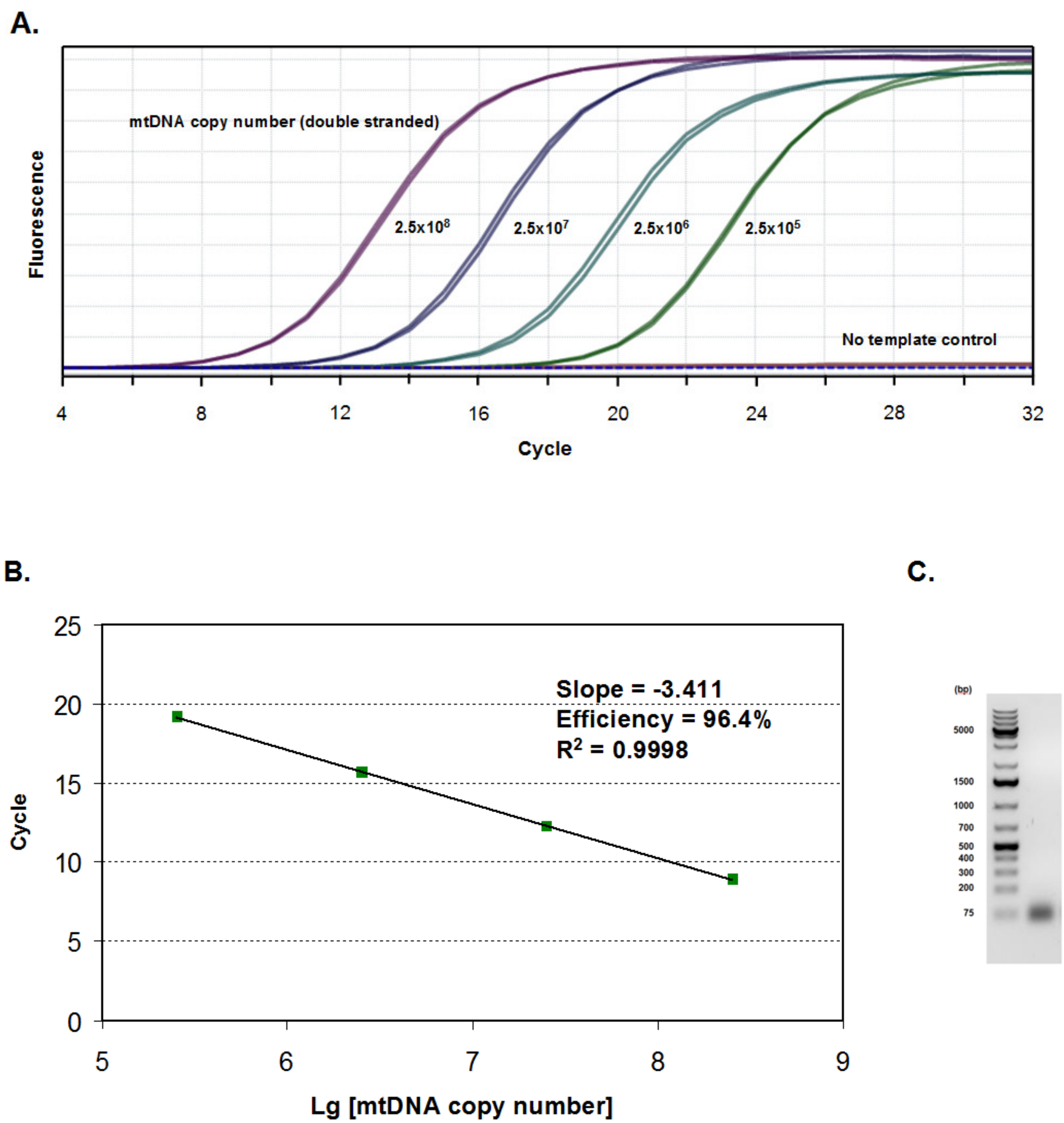


Figure 3. Quality assessment of mtDNA primer set. (**A**) qPCR amplification curves using serially diluted mtDNA template. (**B**) Derivation of qPCR efficiency of mtDNA primer set. **(C)** Separation of mtDNA qPCR product by gel electrophoresis.

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

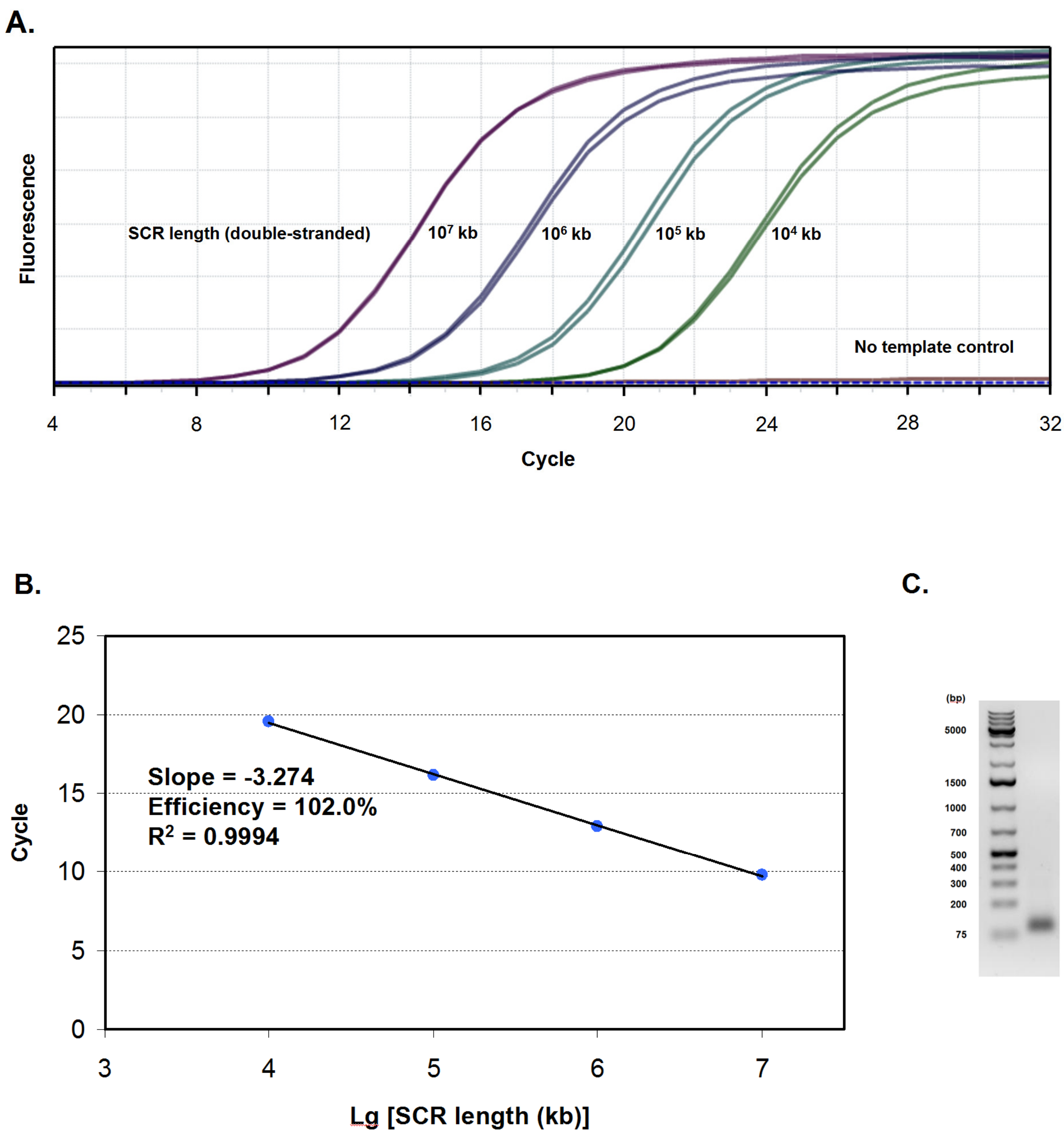


Figure 4. Quality assessment of Single copy reference (SCR) primer set. (A) qPCR amplification curves using serially diluted SCR template. **(B)** Derivation of qPCR efficiency of SCR primer set. **(C)** Separation of SCR qPCR product by gel electrophoresis.

Appendix 3: Method for quantifying reference genomic DNA sample (Cat #8948d)

To quantify the reference genomic DNA sample (Cat #8948d), a qPCR analysis using it as the template was performed. All experiments were performed in triplicates under the same conditions and repeated at least twice.

Derived from the standard curves in appendices 1 and 2, the mtDNA and SCR copy number of reference genomic DNA sample in each qPCR reaction is determined to be:

Total mtDNA copy number (double-stranded): $(1.65 \pm 0.05) \times 10^5$ copies

Total SCR length (double-stranded): 27.6 ± 0.4 kb

The SCR template is 100 bp long, therefore, there are 0.2 kb SCR per diploid cell.

Total number of diploid cells = $(27.6 \pm 0.4 \text{ kb}) / 0.2 \text{ kb} = 138 \pm 2$ cells

mtDNA copy number per diploid cell (double-stranded)

$$= (1.65 \pm 0.05) \times 10^5 \text{ copies} / (138 \pm 2)$$
$$= (1.20 \pm 0.04) \times 10^3 \text{ copies}$$

Conclusions: The average mtDNA copy number of reference genomic DNA sample (Cat #8948d) is $(1.20 \pm 0.04) \times 10^3$ copies per diploid cell.