Rev.1

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

Catalog #8968
50 reactions

Product Description

Circulating mitochondrial DNA (cir-mtDNA), also called cell-free circulating mitochondrial DNA, refers to small fragments of mitochondrial DNA (mtDNA) released by cells under stress or during various detrimental or pathological conditions. Recently, cir-mtDNA content has gained significant traction as a biomarker for assessing mitochondrial function. It is increasingly employed in the study of changes associated with diseases such as cardiovascular and neurodegenerative disorders, utilizing blood samples and other easy-to-collect specimens such as saliva from large populations.

ScienCell's Absolute Human cir-mtDNA Copy Number Quantification qPCR Assay Kit (AHCQ) is designed to quantify the absolute cir-mtDNA copy number of a human plasma/serum sample. 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 reference mitochondrial DNA sample with a known mtDNA copy number per microliter (µL) serves as a reference for cir-mtDNA of target samples. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) no non-specific amplification. mtDNA 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
8968a	Human mtDNA primer set, lyophilized	1 vial	-20°C
8968b	Nuclease-free H_2O	4 mL	4°C
8968c	Reference human mtDNA sample (Lot #38224, mtDNA copy number: 1.3 × 10^6 copies /µL)	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Product Name
cfDNA isolation kit	QIAamp MinElute ccfDNA Midi Kit (50), Cat. No.55284
cir-mtDNA template	Customers' samples
qPCR plate or tube	

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 mtDNA primer sets is validated by template serial dilution (See **Appendices 1**). The copy number of reference mtDNA sample per μL is determined by the qPCR standard curve method.

Product Use

8968 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 #8968a) and the reference genomic DNA sample (Cat #8968c) at -20°C in a manual defrost freezer, and the nuclease-free H_2O (Cat #8968b) 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.

References

[1] Picard M. Blood mitochondrial DNA copy number: What are we counting?. Mitochondrion. 2021 Sep 1;60:1-1.

[2] Rosa HS, Ajaz S, Gnudi L, Malik AN. A case for measuring both cellular and cell-free mitochondrial DNA as a disease biomarker in human blood. The FASEB Journal. 2020 Sep;34(9):12278-88.

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#8968a) 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#8968b) to the mtDNA primer set (lyophilized, Cat #8968a) to make the mtDNA primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
4. For the reference mtDNA sample (Cat#8968c), prepare 20 µl qPCR reactions for one well as shown in Table 1.

Table 1.

Reference mtDNA sample	2 µl
Primer stock solution (mtDNA)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8968b)	6 µl
Total volume	***20 µl***

5. For each cir-mtDNA sample, prepare 20 µl qPCR reactions for one well as shown in Table 2.

Table 2.

cir-mtDNA template (0.5-5 ng/µL)	2 µl
Primer stock solution (mtDNA)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8968c)	6 µl
Total volume	***20 µl***

6. 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).
7. 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 mtDNA sets (Cat #8968a), 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.

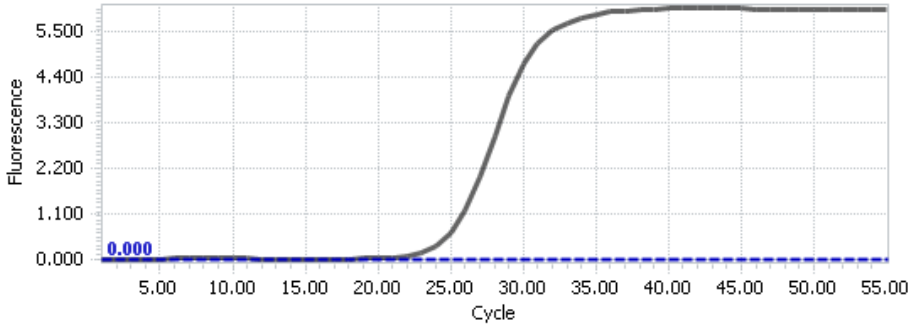
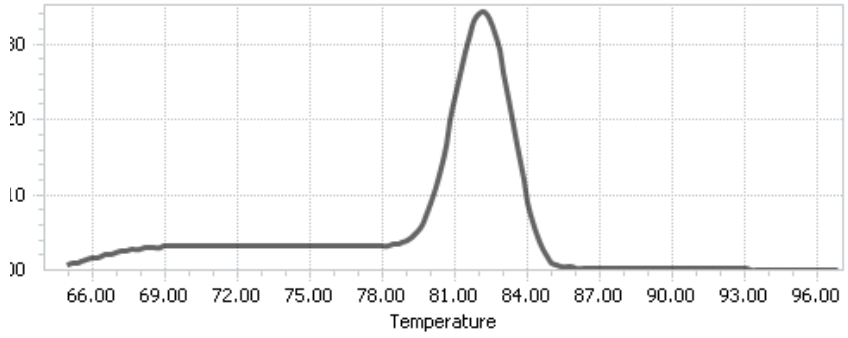


Figure 2. A typical melting peak of a qPCR product.



Quantification Method: Comparative ΔCq (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 cir-mtDNA, ΔCq (cir-mtDNA) is the quantification cycle number difference of mtDNA between the target plasma/serum and the reference mtDNA samples.

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

   Note: The value of ΔCq (cir-mtDNA) can be positive, 0, or negative.

2. Relative cir-mtDNA copy number of the target plasma/serum sample to the reference sample (fold)

   $= 2^{-\Delta Cq}$

3. The cir-mtDNA copy number of the target sample

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

Example Calculations: Comparative ΔCq (Quantification Cycle Value) Method

Table 3. Cq (Quantification Cycle) values of cir-mtDNA mtDNA qPCR obtained for the genomic DNA samples.

Primer set	*Target sample*	*Reference sample*
cir-mtDNA	25.89	18.8

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

= 25.89 – 18.8

= 7.09

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

$= 2^{-\Delta Cq}$

$= 2^{-7.09}$

= 0.00734

The cir-mtDNA copy number of the target sample per 2 µL

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

= 1.3 × 10^6 x 0.00734

= 954

The cir-mtDNA copy number of the target sample per 1 μL
= 954/2
= 477

Example Conclusions: The average cir-mtDNA of the target plasma/serum sample is 477 copies per μL.

Appendix 1: Quality assessment of mtDNA primer set

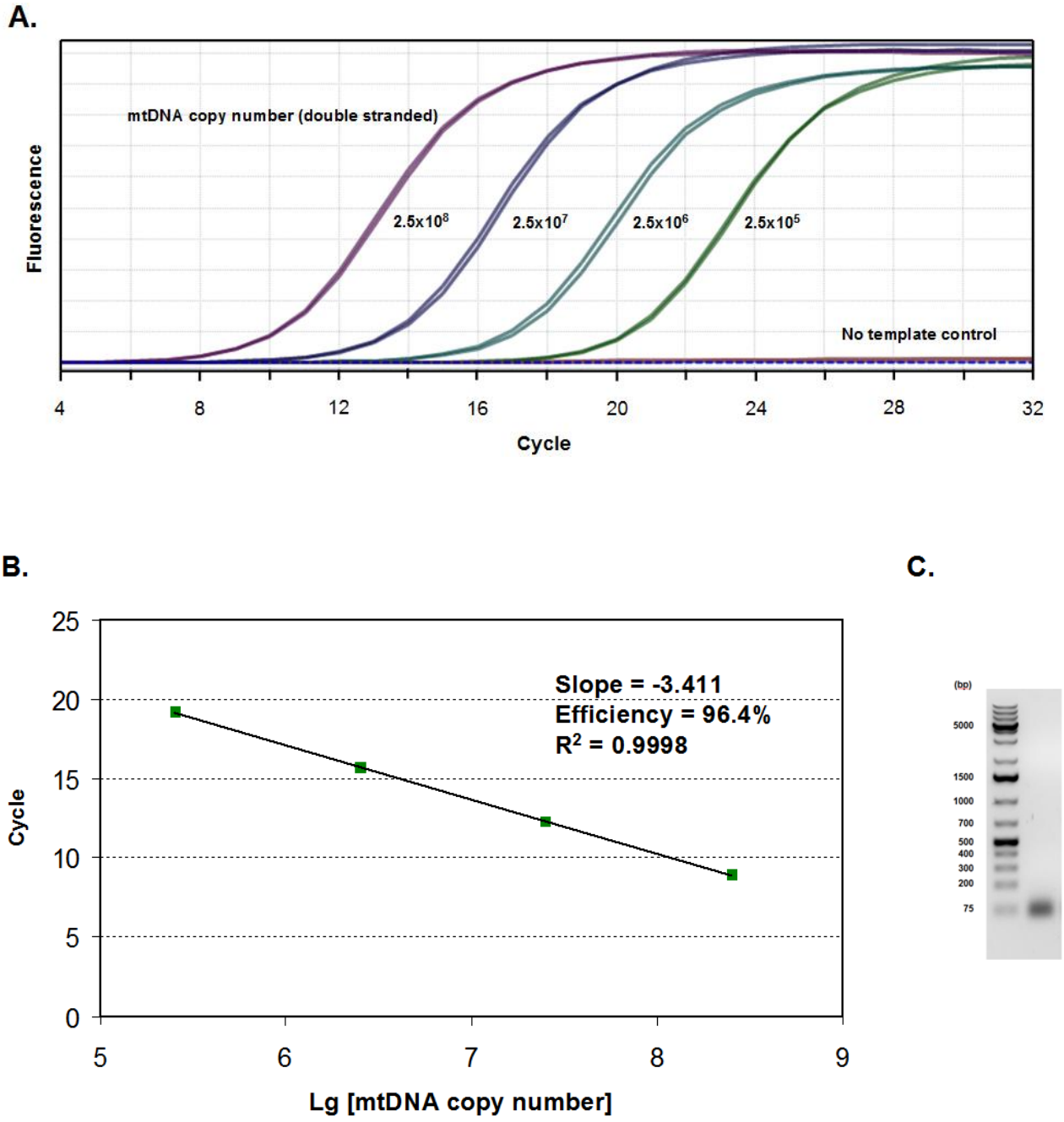


Figure 1. Quality assessment of mtDNA primer set. (A) qPCR amplification curves using serially diluted mtDNA template. **(B)** Derivation of qPCR efficiency of mtDNA primer set. **(C)** Separation of mtDNA qPCR product by gel electrophoresis.