Human Age-associated ELOVL2 Promoter Methylation Quantification qPCR Assay Kit (HAEPM)

Catalog #8998
100 reactions

Product Description

DNA methylation at CpG islands in promoters regulates gene expression. It is a dynamic epigenetic process affected by various genetic and environmental factors including aging. The CpG island methylation level at human ELOVL2 gene promoter has been well-associated with chronological age in various populations, cell types, and tissues. ELOVL2 promoter methylation level is therefore a potential marker for human chronological age.

ScienCell's Human Age-associated ELOVL2 Promoter Methylation Quantification qPCR Assay Kit (HAEPM) is designed to quantify the level of human ELOVL2 promoter methylation. The reference DNA sample consists of a 1:1 ratio of methylated ELOVL2 promoter copies to non-methylated ELOVL2 promotor copies (both copies represent bisulfite converted promoter sequences), and serves as a reference for calculating the ratio of methylated to non-methylated ELOVL2 promotor of target samples. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) negligible 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	Quantity	Storage
8998a	Methylated ELOVL2 promoter (MEP) primer set, lyophilized	1 vial	-20°C
8998b	Non-methylated ELOVL2 promoter (OEP) primer set, lyophilized	1 vial	-20°C
8998c	Nuclease-free H_2O	4 mL	4°C
8998d	Reference DNA sample	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)
bisulfite conversion kit	EpiTect Bisulfite Kits (Qiagen, Cat #59104)
qPCR plate or tube	
qPCR master mix	FastStart Essential DNA Green Master (Roche, Cat #06402712001)

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 reference DNA sample is sequenced by Sanger sequencing.

Product Use
HAEPM 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 #8998a and 8998b) and the reference DNA sample (Cat #8998d) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #8998c) at 4°C.

Procedures

Important: ***Only*** *use Taq DNA polymerase-based qPCR master mix, as Pfu DNA polymerase can NOT amplify uracil-containing templates.* ***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. HAEPM 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. This kit works ONLY with bisulfite converted genomic DNA samples. For genomic DNA isolation and bisulfite conversion, we recommend using DNeasy Blood & Tissue Kit (Qiagen, Cat #69504, 69506) and EpiTect Bisulfite Kits (Qiagen, Cat #59104), respectively. Please follow manufacturer's instructions to obtain bisulfite converted genomic DNA samples.

2. Prior to use, allow vials (Cat #8998a and #8998b) to warm to room temperature. Centrifuge the vials at 1,500x g for 1 minute.

3. Add 200 µl nuclease-free H_2O (Cat #8998c) to MEP primer set (lyophilized, Cat #8998a) to make MEP 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 #8998c) to OEP primer set (lyophilized, Cat #8998b) to make OEP 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 #8998d), prepare two qPCR reactions, one with MEP primer stock solution, and one with OEP primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 1.

Table 1.

Reference DNA sample (Cat #8998d)	1 µl
Primer stock solution (MEP or OEP)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #8998c)	7 µl
Total volume	***20 µl***

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

Table 2.

Bisulfite converted genomic DNA sample	5-20 ng
Primer stock solution (MEP or OEP)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #8998c)	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 MEP and OEP primer sets (Cat #8998a and #8998b), we highly recommend an annealing temperature of 60°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	60°C	20 sec	
Extension	72°C	20 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.

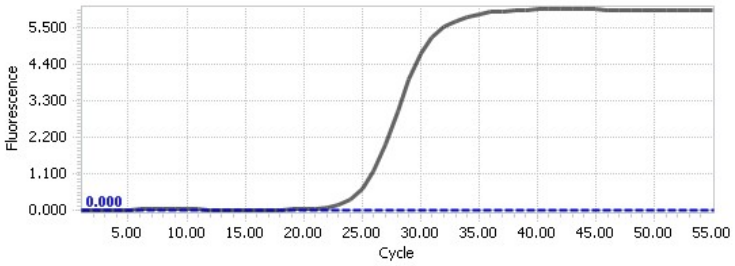
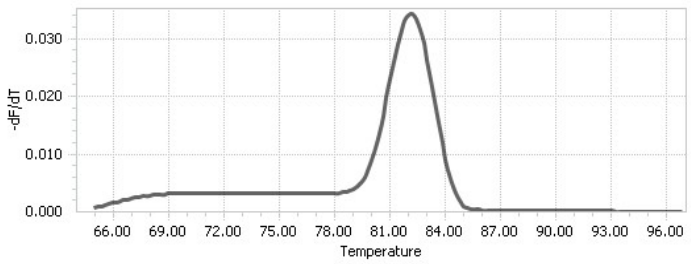


Figure 2. A typical melting peak of a qPCR product.



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 methylated ELOVL2 promoter (MEP), ΔCq (MEP) is the quantification cycle number difference of MEP between the target and the reference DNA samples.

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

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

2. For non-methylated ELOVL2 promoter (OEP), ΔCq (OEP) is the quantification cycle number difference of OEP between the target and the reference DNA samples.

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

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

3. $\Delta\Delta Cq = \Delta Cq\ (MEP) - \Delta Cq\ (OEP)$

4. The ratio of MEP to OEP of the target sample = $2^{-\Delta\Delta Cq}$

5. The percentage of methylated ELOVL2 promoter $= 2^{-\Delta\Delta Cq} / (2^{-\Delta\Delta Cq} + 1) \times 100\%$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 4. Cq (quantification cycle) values obtained for the samples by qPCR using MEP and OEP primer sets.

Primer set	*Target sample*	*Reference sample*
MEP	25.08	20.99
OEP	25.64	20.80

ΔCq (MEP) = Cq (MEP, target sample) - Cq (MEP, reference sample)
= 25.08 - 20.99
= 4.09

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

ΔΔCq = ΔCq (MEP) - ΔCq (OEP)
= 4.09- (4.84)

$= -0.75$

The ratio of MEP to OEP of the target sample

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

$= 2^{0.75}$

$= 1.7$

The percentage of methylated ELOVL2 promoter

$= 1.7/(1.7+1) \times 100\%$

$= 63\%$

Conclusions: The percentage of methylated ELOVL2 promoter in the target sample is 63%.

Appendix 1: Quality assessment of methylated ELOVL2 promoter (MEP) primer set

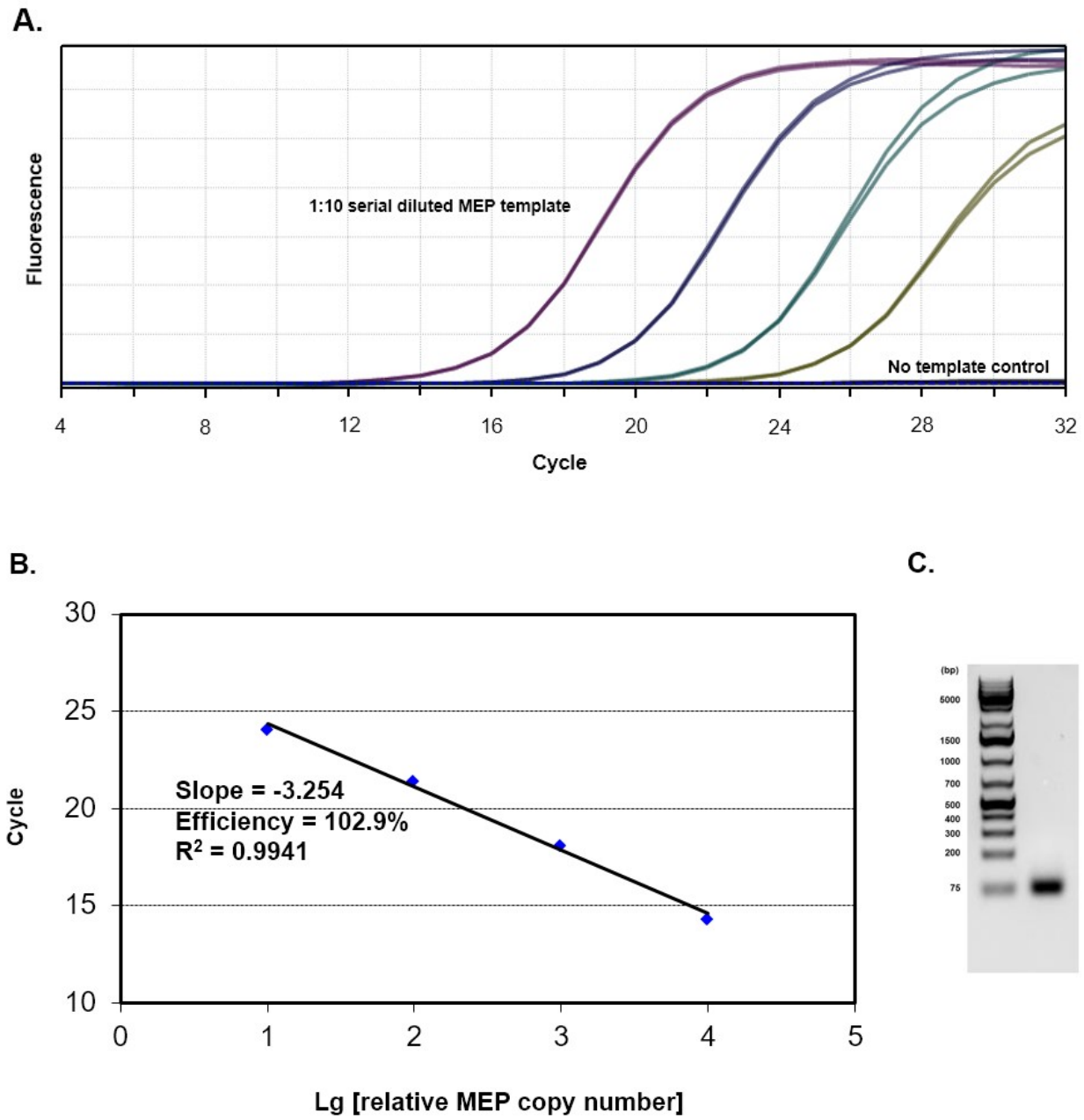


Figure 3. Quality assessment of MEP primer set. (A) qPCR amplification curves using serially diluted MEP repeats as template. **(B)** Derivation of qPCR efficiency of MEP primer set. **(C)** Separation of MEP qPCR product by gel electrophoresis.

Appendix 2: Quality assessment of non-methylated ELOVL2 promoter (OEP) primer set

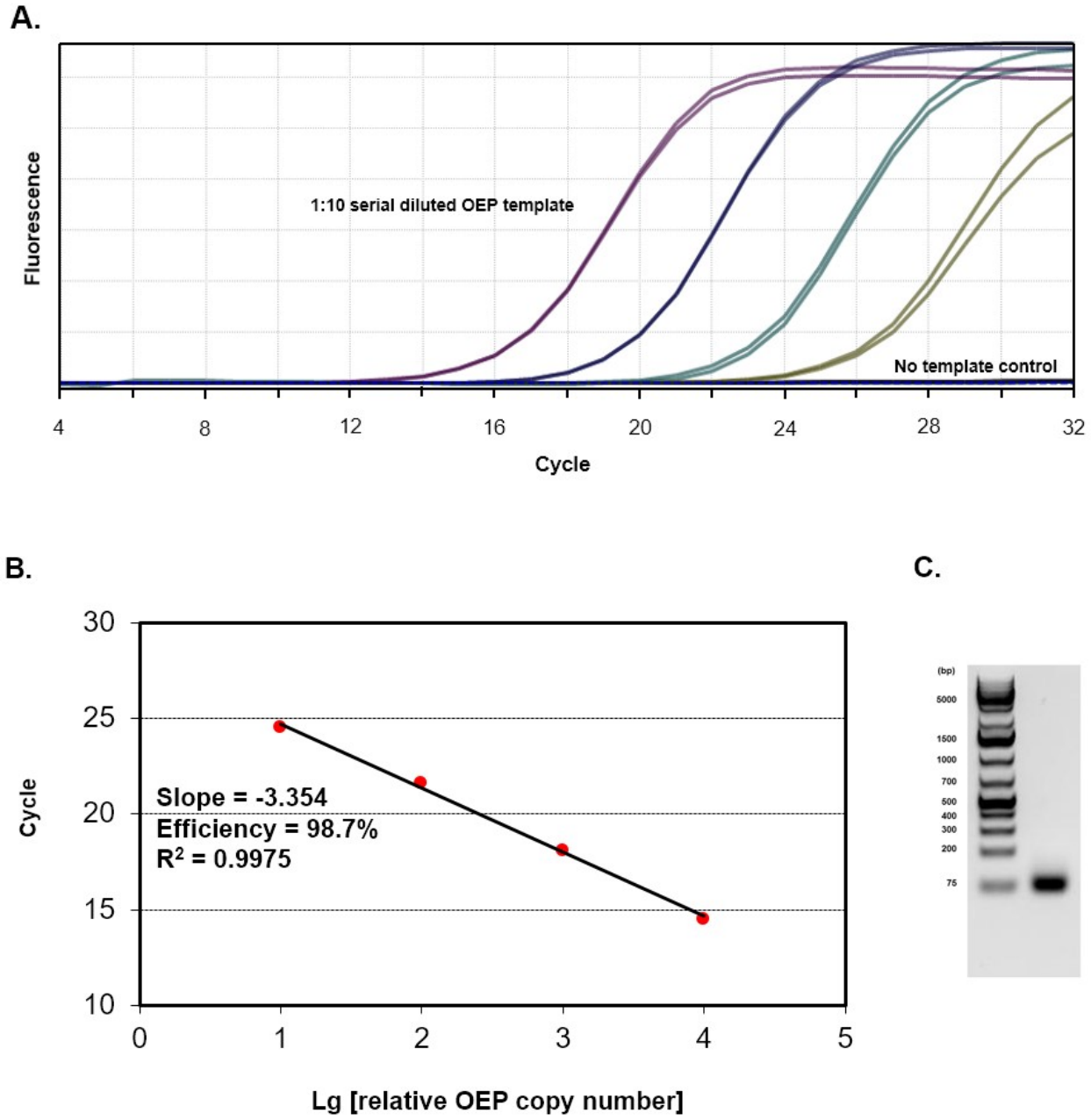


Figure 4. Quality assessment of Single copy reference (OEP) primer set. (A) qPCR amplification curves using serially diluted OEP template. **(B)** Derivation of qPCR efficiency of OEP primer set. **(C)** Separation of OEP qPCR product by gel electrophoresis.