



Human Global DNA Methylation Quantification qPCR Assay Kit (HGDM)

Catalog #7008

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. Numerous diseases including cancer have been linked to aberrant global changes in genomic DNA methylation. It is generally thought that global genomic DNA methylation level can be represented by the methylation level at repetitive elements. One example of this includes the repetitive Long Interspersed Nuclear Element-1 (LINE-1), which comprises 17-18% of the human genome and is usually heavily methylated in most normal primary cells.

ScienCell's Human Global DNA Methylation Quantification qPCR Assay Kit (HGDM) is designed to quantify the level of human LINE1 promoter methylation. The reference DNA sample consists of a 1:1 ratio of methylated LINE1 promoter copies to non-methylated LINE1 promoter copies (both copies represent bisulfite converted promoter sequences), and serves as a reference for calculating the ratio of methylated to non-methylated LINE1 promoter 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
7008a	Methylated LINE1 promoter (MLP) primer set, lyophilized	1 vial	-20°C
7008b	Non-methylated LINE1 promoter (OLP) primer set, lyophilized	1 vial	-20°C
7008c	Nuclease-free H ₂ O	4 mL	4°C
7008d	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

HGDM 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 #7008a and 7008b) and the reference DNA sample (Cat #7008d) at -20°C in a manual defrost freezer, and nuclease-free H₂O (Cat #7008c) 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. HGDM 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 #7008a and #7008b) to warm to room temperature. Centrifuge the vials at 1,500x g for 1 minute.
3. Add 200 μ l nuclease-free H₂O (Cat #7008c) to MLP primer set (lyophilized, Cat #7008a) to make MLP primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
4. Add 200 μ l nuclease-free H₂O (Cat #7008c) to OLP primer set (lyophilized, Cat #7008b) to make OLP 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 #7008d), prepare two qPCR reactions, one with MLP primer stock solution, and one with OLP primer stock solution. Prepare 20 μ l qPCR reactions for one well as shown in Table 1.

Table 1.

Reference DNA sample (Cat #7008d)	1 μ l
Primer stock solution (MLP or OLP)	2 μ l
2x qPCR master mix	10 μ l
Nuclease-free H ₂ O (Cat #7008c)	7 μ l
Total volume	20 μl

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

Table 2.

Bisulfite converted genomic DNA sample	5-20 ng
Primer stock solution (MLP or OLP)	2 μ l
2x qPCR master mix	10 μ l
Nuclease-free H ₂ O (Cat #7008c)	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 MLP and OLP primer sets (Cat #7008a and #7008b), 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		
<i>Optional</i>	<i>Melting curve analysis</i>		1
Hold	20°C	Indefinite	1

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

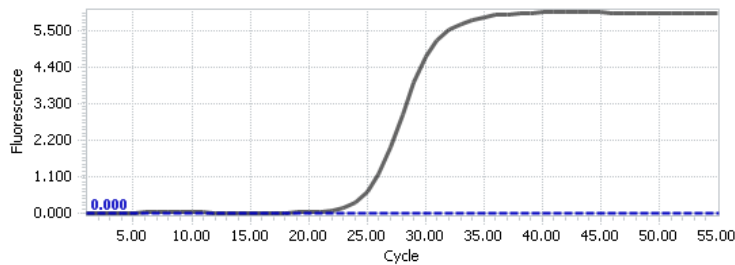
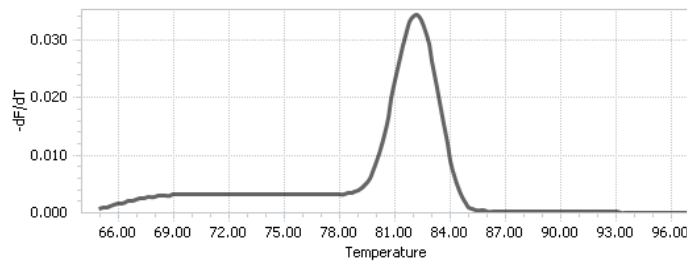


Figure 2. A typical melting peak of a qPCR product.



Quantification Method: Comparative $\Delta\Delta Cq$ (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 LINE1 promoter (MLP), ΔCq (MLP) is the quantification cycle number difference of MLP between the target and the reference DNA samples.

$$\Delta Cq \text{ (MLP)} = Cq \text{ (MLP, target sample)} - Cq \text{ (MLP, reference sample)}$$

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

2. For non-methylated LINE1 promoter (OLP), ΔCq (OLP) is the quantification cycle number difference of OLP between the target and the reference DNA samples.

$$\Delta Cq \text{ (OLP)} = Cq \text{ (OLP, target sample)} - Cq \text{ (OLP, reference sample)}$$

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

3. $\Delta\Delta Cq = \Delta Cq \text{ (MLP)} - \Delta Cq \text{ (OLP)}$
4. The ratio of MLP to OLP of the target sample = $2^{-\Delta\Delta Cq}$
5. The percentage of methylated LINE1 promoter = $2^{-\Delta\Delta Cq} / (2^{-\Delta\Delta Cq} + 1) \times 100\%$

Example Calculations: Comparative $\Delta\Delta Cq$ (Quantification Cycle Value) Method

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

<i>Primer set</i>	<i>Target sample</i>	<i>Reference sample</i>
MLP	24.74	21.33
OLP	23.98	21.51

$$\begin{aligned}\Delta Cq \text{ (MLP)} &= Cq \text{ (MLP, target sample)} - Cq \text{ (MLP, reference sample)} \\ &= 24.74 - 21.33 \\ &= 3.41\end{aligned}$$

$$\begin{aligned}\Delta Cq \text{ (OLP)} &= Cq \text{ (OLP, target sample)} - Cq \text{ (OLP, reference sample)} \\ &= 23.98 - 21.51 \\ &= 2.47\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq &= \Delta Cq \text{ (MLP)} - \Delta Cq \text{ (OLP)} \\ &= 3.41 - (2.47)\end{aligned}$$

$$= 0.94$$

The ratio of MLP to OLP of the target sample

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

$$= 2^{-0.94}$$

$$= 0.52$$

The percentage of methylated LINE1 promoter

$$= 0.52/(0.52+1) \times 100\%$$

$$= 34\%$$

Conclusions: The percentage of methylated genomic DNA in the target sample is 34%.

Appendix 1: Quality assessment of methylated LINE1 promoter (MLP) primer set

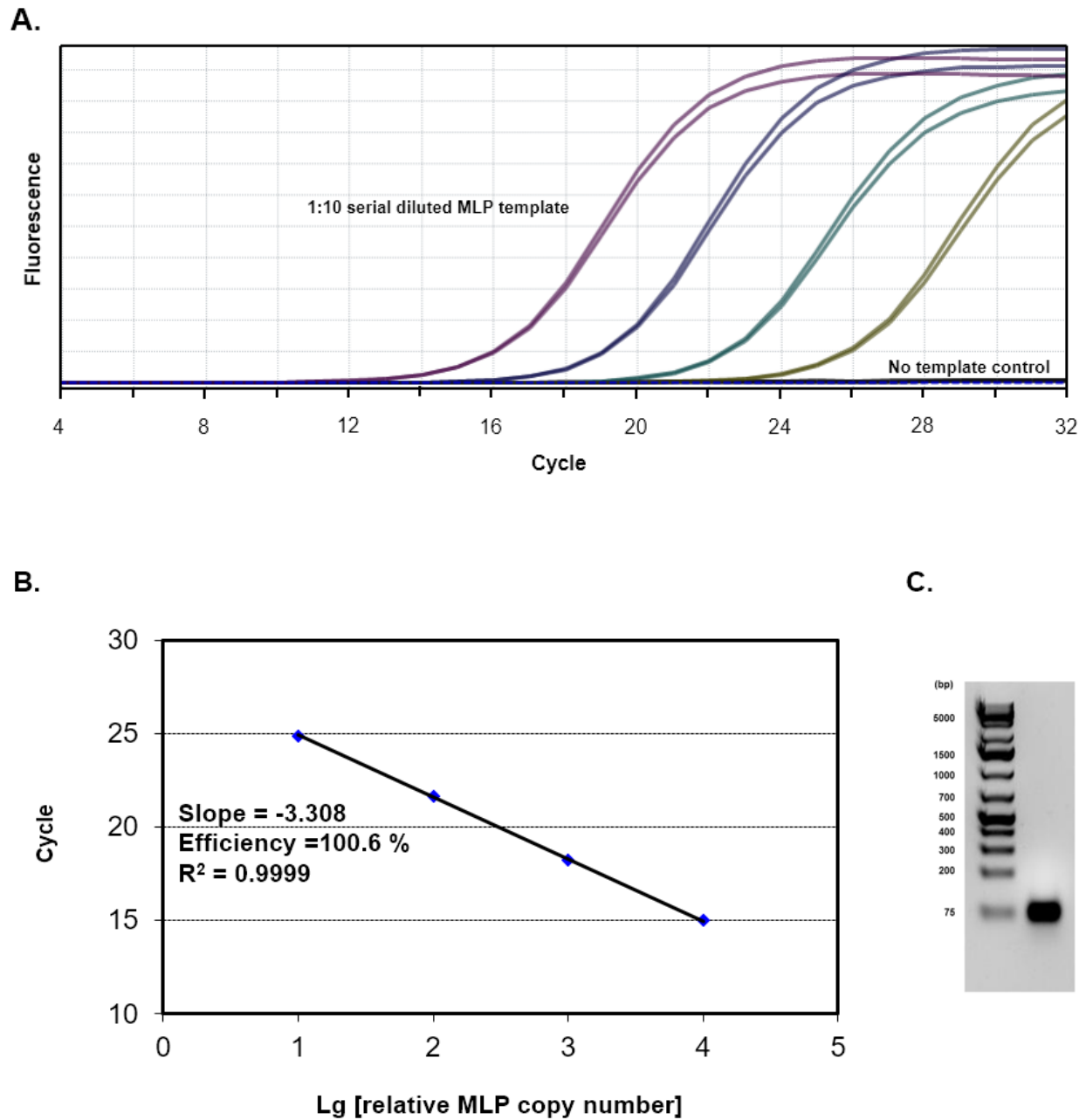


Figure 3. Quality assessment of MLP primer set. (A) qPCR amplification curves using serially diluted MLP repeats as template. (B) Derivation of qPCR efficiency of MLP primer set. (C) Separation of MLP qPCR product by gel electrophoresis.

Appendix 2: Quality assessment of non-methylated LINE1 promoter (OLP) primer set

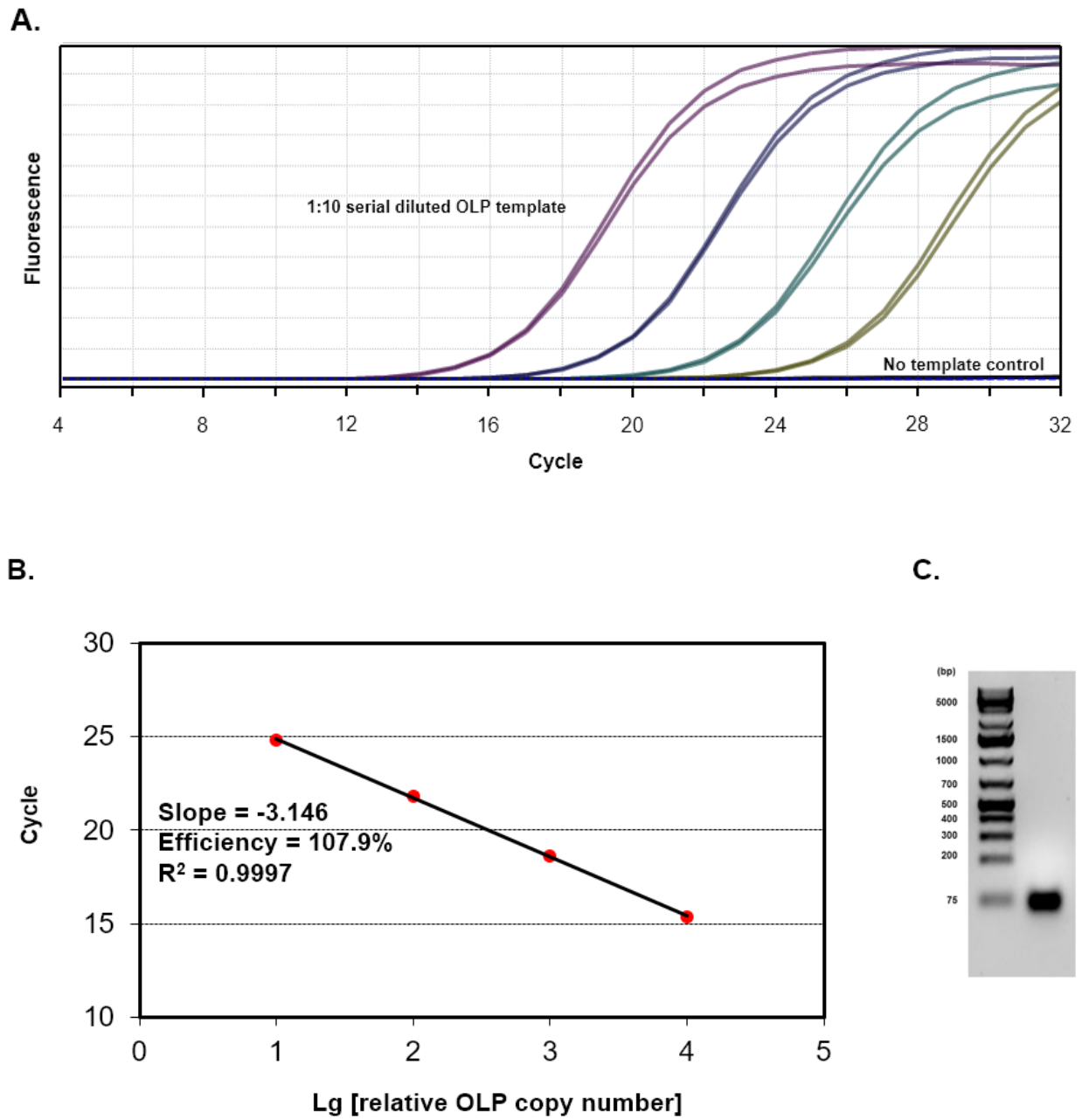


Figure 4. Quality assessment of Single copy reference (OLP) primer set. (A) qPCR amplification curves using serially diluted OLP template. **(B)** Derivation of qPCR efficiency of OLP primer set. **(C)** Separation of OLP qPCR product by gel electrophoresis.