Human Cancer Promoter Methylation qPCR Assay Kit (HCPM)

Catalog #9058
50 reactions

Product Description

DNA methylation at CpG islands within gene promoter regions plays a key role in the regulation of gene expression. Aberrant promoter methylation, a common epigenetic alteration in cancer, often leads to the silencing of tumor suppressor genes such as RASSF1A, GSTP1, and CDKN2A/p16, which are among the most frequently methylated in human cancers. Hypermethylation of these genes has been implicated in various tumor types and is considered a valuable biomarker for cancer detection, prognosis, and therapeutic monitoring. ScienCell's Human Cancer Promoter Methylation qPCR Assay Kit (HCPM) is designed to accurately quantify the methylation levels of RASSF1A, GSTP1, and CDKN2A/p16 promoters using bisulfite-converted DNA. The included reference DNA sample contains a 1:1 ratio of methylated to non-methylated promoter sequences for each target gene, serving as a calibration standard for quantifying methylation levels in unknown samples. The assay features highly specific primers that distinguish between methylated and non-methylated DNA, validated through qPCR melt curve analysis, gel electrophoresis, and serial dilution to ensure amplification specificity and efficiency.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2X GoldNStart TaqGreen qPCR master mix, 1mL	3 vials	-20°C
9058a	Methylated RASSF1A promoter (MRP) primer set	1 vial	-20°C
9058b	Non-methylated RASSF1A promoter (ORP) primer set	1 vial	-20°C
9058c	Methylated GSTP1 promoter (MGP) primer set	1 vial	-20°C
9058d	Non-methylated GSTP1 promoter (OGP) primer set	1 vial	-20°C
9058e	Methylated CDKN2A/p16 promoter (MCP) primer set	1 vial	-20°C
9058f	Non-methylated CDKN2A/p16 promoter (OCP) primer set	1 vial	-20°C
9058g	Nuclease-free H_2O	4 mL	4°C
9058h	Reference DNA sample (Methylated: Non-methylated= 1:1)	100 µL	-20°C

Notes: All primers are provided lyophilized; primer codes starting with 'M' indicate methylated sequences, while codes starting with 'O' indicate non-methylated sequences.

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
DNA isolation kit	SpeeDNA Isolation Kit (Cat #MB6918)
Bisulfite conversion kit	EZ DNA Methylation-Gold (Zymo Research, Cat # D5005)
qPCR plate or tube	N/A

Quality Control
All primers set are validated by qPCR with melt curve analysis. The efficiency of the primer sets is validated by template serial dilution and gel electrophoresis (See **Appendices 1 and 2**).

Product Use
HCPM 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 #9058a to f) and the reference DNA sample (Cat #9058h) at -20°C in a manual defrost freezer, and the nuclease-free H2O (Cat #9058c) at 4°C. Once thawed, do NOT refreeze GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1), and always keep in the dark at -20°C or on ice.

Procedures
This kit works ONLY with bisulfite converted genomic DNA samples. For genomic DNA isolation and bisulfite conversion, we recommend using SpeeDNA Isolation Kit (Cat #MB6918) and EZ DNA Methylation-Gold (Zymo Research, Cat #D5005), respectively. Please follow manufacturer's instructions to obtain bisulfite converted genomic DNA samples.

1. Prior to use, allow vials (Cat #9058a, #9058b, #9058c, #9058d, #9058e, and #9058f) to warm to room temperature. Centrifuge the vials at 1,500x g for 1 minute.

2. Add 200 µl nuclease-free H_2O (Cat#9058g) to methylated and non-methylated primer sets (lyophilized, Cat #9058a, #9058b, #9058c, #9058d, #9058e, #9058f) to make primer stock solutions. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

3. For the reference DNA sample (Cat #9058h), prepare six qPCR reactions: one each with MRP, ORP, MGP, OGP, MCP, and OCP primer stock solutions. Set up 20 µl qPCR reactions for one well as shown in Table 1.

   Table 1.

Reference DNA sample (Cat #9058h)	2 µl
Primer stock solution (MRP, ORP, MGP, OGP, MCP or OCP)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #9058g)	6 µl
Total volume	***20 µl***

4. For each bisulfite-converted genomic DNA sample, prepare six qPCR reactions: one each with MRP, ORP, MGP, OGP, MCP, and OCP primer stock solutions. Set up 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 (MRP, ORP, MGP, OGP, MCP or OCP)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #)	variable
Total volume	***20 µl***

5. 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).

6. 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 the primer sets (Cat#9058b-#9058f), we highly recommend an annealing temperature of 58°C as shown in Table 3:

Table 3.

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	30 sec	40
Annealing	58°C	30 sec	
Extension	72°C	30 sec	
Data acquisition	Plate read		
Optional	*Melting curve analysis*		1

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

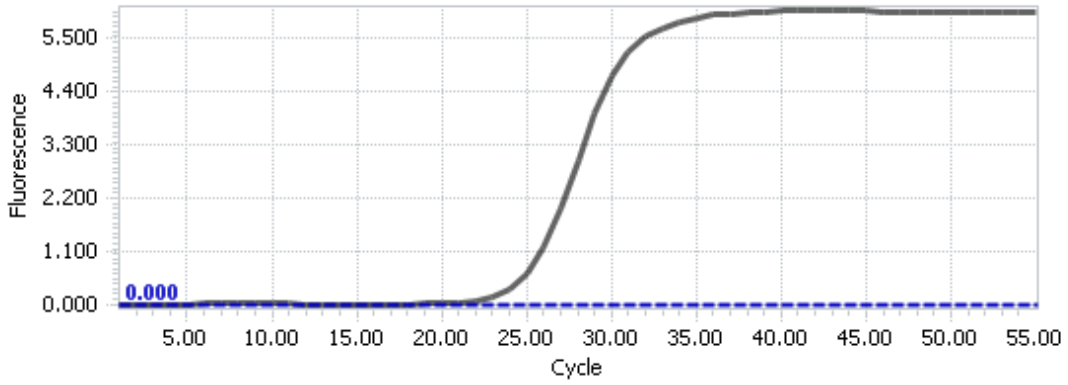
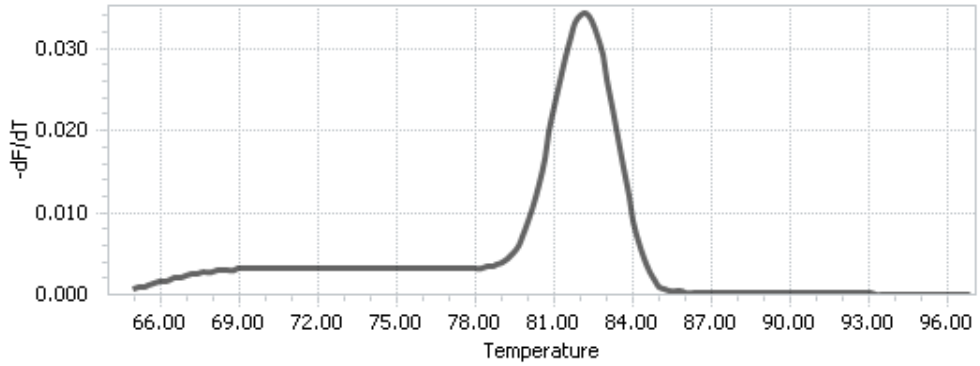


Figure 2. A typical melting peak of a qPCR product.



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 RASSF1A promoter (MRP), ΔCq (MRP) is the quantification cycle number difference of MRP between the target and the reference DNA samples.

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

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

2. For non-methylated RASSF1A promoter (ORP), ΔCq (ORP) is the quantification cycle number difference of ORP between the target and the reference DNA samples.

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

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

3. $\Delta\Delta Cq,e = \Delta Cq$ (MRP) - ΔCq (ORP)

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

5. The percentage of methylated RASSF1A promoter $=2^{-\Delta\Delta Cq} / (2^{-\Delta\Delta Cq} + 1) \times 100\%$

Note: The same procedure applies for GSTP1 (MGP vs OGP) and CDKN2A/p16 (MCP vs OCP).

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 4. Cq (quantification cycle) values obtained for the samples by qPCR using MRP, ORP, MGP, OGP, MCP and OCP primer sets.

Primer set	Target sample	Reference sample
MRP	27.59	23.49
ORP	26.63	22.12
MGP	26.09	21.21
OGP	26.90	20.91
MCP	27.50	19.11
OCP	26.00	18.93

Detailed example (RASSF1A):

- ΔCq (MRP) = Cq (MRP, target sample) - Cq (MRP, reference sample)
  = 27.59 – 23.49
  = 4.1

  ΔCq (ORP) = Cq (ORP, target sample) - Cq (ORP, reference sample)
  = 26.63 – 22.12
  = 4.51

  ΔΔCq = ΔCq (MRP) - ΔCq (ORP)
  = 4.1- (4.51)
  = -0.41

  The ratio of MRP to ORP of the target sample
  $= 2^{-\Delta\Delta Cq}$
  $= 2^{0.41}$
  = 1.33

  The percentage of methylated RASSF1A promoter
  = 1.33/ (1.33+1) x 100%
  = 57%

Note: Results for the other genes (calculated using the same method):

- GSTP1 promoter: 68% methylated
- CDKN2A/p16 promoter: 76% methylated

Conclusions: The percentage of methylated *RASSF1A* promoter in the target sample is 57%, the percentage of methylated *GSTP1* in the target sample is 68% and, the percentage of methylated *CDKN2A/p16* promoter in the target sample is 76%.

Appendix 1: Quality assessment of methylated (MCP, MGP, MRP) and Non-methylated (OCP, OGP, ORP) primer sets for CDKN2A/p16, GSTP1, and RASSF1A promoters.

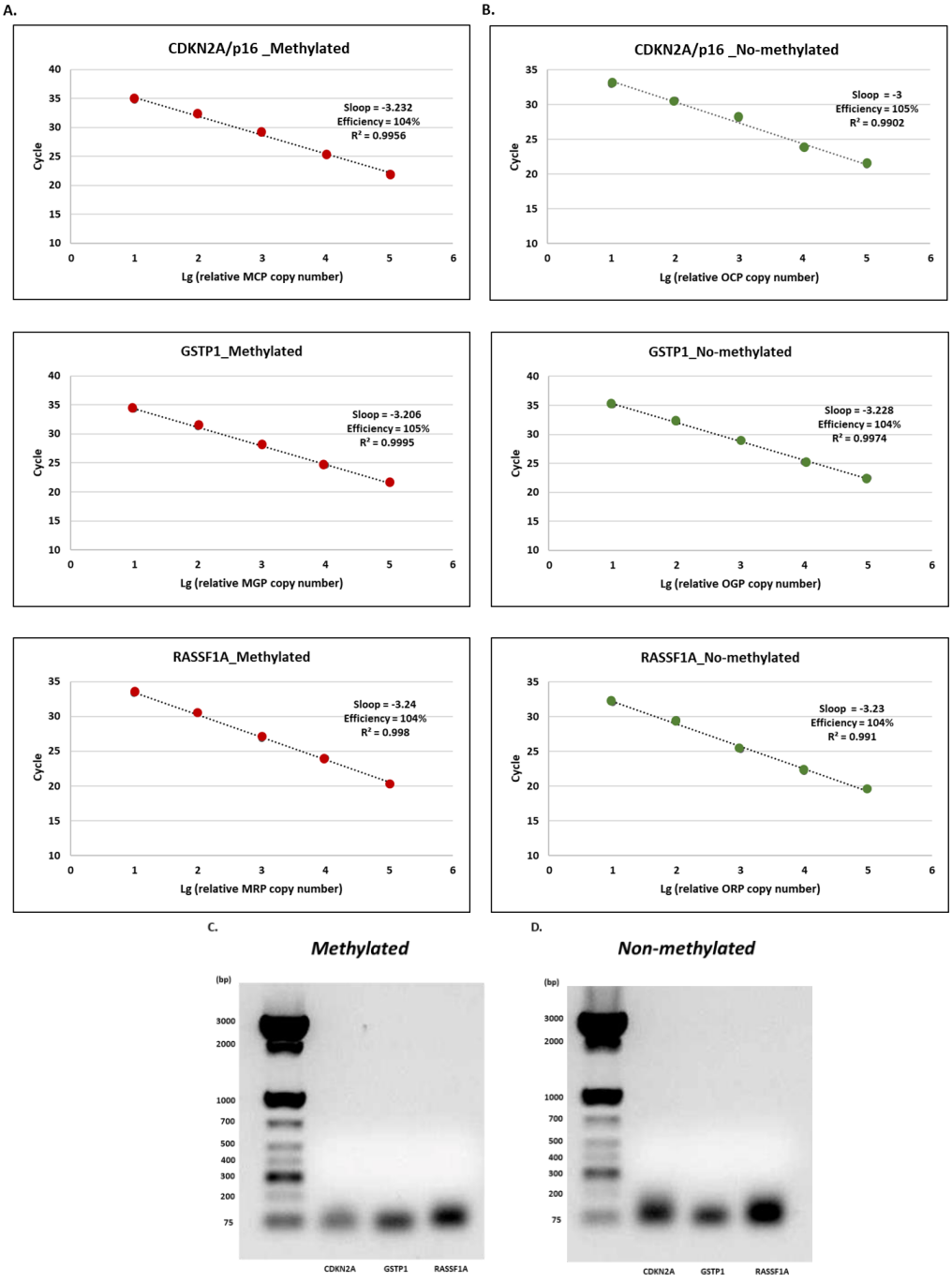


Figure 3. Quality assessment of Methylated and Non-methylated primer sets. (**A**) qPCR efficiency analysis of methylated primer sets (MCP, MGP, and MRP). (**B**) qPCR efficiency analysis of non-methylated primer sets (OCP, OGP, and ORP). (**C**) Separation of MCP, MGP, and MRP qPCR products by agarose gel electrophoresis. (**D**) Separation of OCP, OGP, and ORP qPCR products by agarose gel electrophoresis.