

Rat High Methylated Genomic DNA Control



Product Highlights

- ▶ Rat High Methylated Genomic DNA is derived from liver and *in vitro* enzymatically methylated resulting in greater than 85% methylation
- ▶ Ideal for use as control in bisulfite methylation analysis procedures including Pyrosequencing, targeted NGS, and Methylation Sensitive High-Resolution Melting (MS-HRM)
- ▶ Tested on gene specific and global methylation assays for consistent performance
- ▶ Requires bisulfite modification prior to use

Intended Use: FOR RESEARCH USE ONLY

Product Contents

1 vial Rat High Methylated Genomic DNA (5 µg at 100 ng/µL, > 85% Methylation)

Ordering Information

CATALOG NUMBER	PRODUCT	PRICE
80-8065-RGHM5	Rat high methylated genomic DNA (5 µg at 100 ng/ µL)	\$269.25

Example Protocol

- ▶ Bisulfite modification of Premix and sample(s) of interest.
 - Zymo Research EZ Methylation kit (Cat.#D5002 or D5004) with 200 - 500 ng of input DNA following manufacturers recommended protocol.

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► PCR amplification Protocol

Component	Per 30µl reaction
10X PCR buffer (Contains 15mM MgCl ₂)	3 µL (1x)
25 mM MgCl ₂	1.8 µL (3.0 mM final conc.)
10 mM dNTPs	0.6 µL (200 µM of each)
10 µM Forward primer	0.6 µL (6 pmol)
10 µM Reverse primer	0.6 µL (6 pmol)
HotStart Taq Polymerase (5 U/µl)	0.15 µL (0.75 U)
DNA	1 µL of bisulfite treated DNA
Water	Adjust to 30 µL

- HotStart Taq Polymerase Qiagen Cat. #203205 recommended with the following PCR cycling conditions:
 - 95°C 15 min; 45 x (95°C 30s; T_a°C 30 s; 72°C 30 s); 72°C 5 min; 4°C ∞
 - Additional optimization is needed if different PCR system is used in analysis.
- Sequencing Analysis: Pyrosequencing, NGS, or MS-HRM.

Technical Specifications

- 5µg DNA in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0)
- Store at -20°C, in aliquots, for 2 years. For best results, do not freeze/thaw an individual aliquot more than three times. For longer term storage -70°C is recommended.

Example Quality Control Results

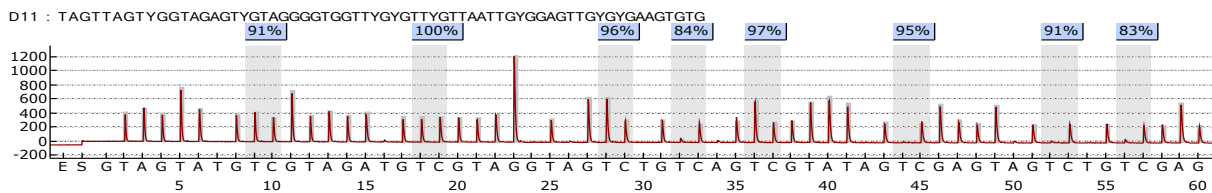


Figure: High Methylated Control DNA Tested on a Rat *NR3C1* Methylation Assay via Pyrosequencing

Pyrogram showing approximately $\geq 90\%$ methylation at all CpG sites

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