

Lambda Phage High Methylated DNA Control



Product Name: Lambda High Methylated DNA Control

Catalog Number: 80-8069-LHM5

Product Contents: 1 vial Lambda Phage High Methylated DNA (5µg at 100 ng/µL, >85% Methylation)

Technical Specifications: 5µg in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) Store at -20°C, upon arrival, 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.

Intended Use: FOR RESEARCH USE ONLY

Example Protocol

- ▶ Bisulfite modification of controls 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.

Product Highlights

- ▶ Lambda Phage High Methylated DNA control is derived commercially from New England BioLabs 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 methylation assay for consistent performance.
- ▶ Requires bisulfite modification prior to use.

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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 30 s; Ta°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.

Example Quality Control Results

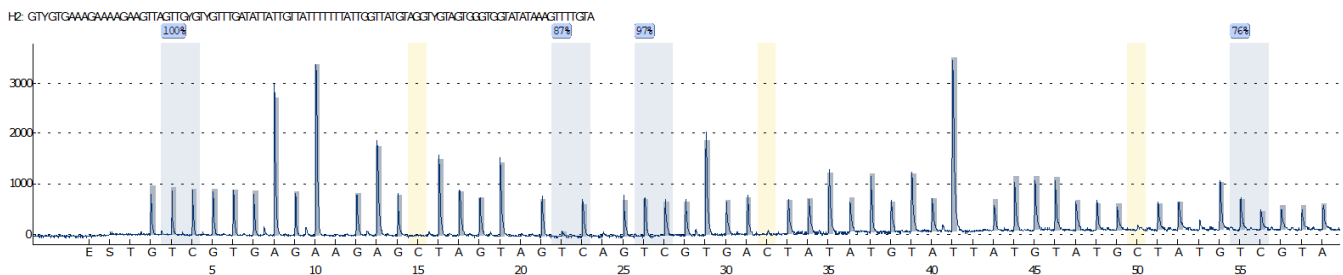


Figure: High Methylated Lambda Control DNA Tested on a Lambda Phage Specific DNA Assay via Pyrosequencing
Pyrogram showing approximately 85% methylation at all CpG sites

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