CpG Methylated HeLa Genomic DNA

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N4007S 0051205 14051

N4007S

15 µg **Lot: 0051205** **Exp: 5/14**

100 µg/ml **Store at –20°C**

Description: Human female HeLa (cervix adenocarcinoma) genomic DNA that was enzymatically methylated with CpG Methylase (M. SssI), suitable as a positive control in the study of CpG dinucleotide methylation.

Source: HeLa (cervix adenocarcinoma) cells were grown to confluency in DMEM plus 10% fetal bovine serum. Genomic DNA was isolated by a standard genomic purification protocol (1), treated with CpG Methylase (M. SssI), phenol extracted and equilibrated to 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA.

Application:

- A positive control for Methylation-Specific PCR (MSP) (2), Bisulfite sequencing, Methylation-sensitive Single-Nucleotide Primer Extension (Ms-SNuPE), Combined Bisulfite Restriciton Analysis (COBRA), Bisulfite treatment and PCR-Single-Strand Conformation Polymorphism Analysis (Bisulfite-PCR-SSCP/BiPS).

Supplied in: 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Avoid multiple freeze/thaw cycles.

Quality Assurance: Purified free of contaminating proteins and RNA.

$A_{260/280}$ Ratio: 1.87

Quality Control Assays

Bisulfite conversion followed by Methylation-Specific PCR (MSP): 10 µl (1 µg) of CpG methylated HeLa genomic DNA were bisulfite converted (3) and eluted in 40 µl of TE buffer. 5 µl were added to a 20 µl PCR reaction containing primers specific to fully CpG methylated PTEN or Rb promoter DNA. A control set of primers designed to anneal to unmethylated PTEN or Rb promoter DNA were also used. Only the methylated-specific primer sets generated the appropriate sized PCR product.

S-adenosyl-L-[methyl-^{3}H] methionine (AdoMet) Incorporation Assay: Incubation of 1 µg of CpG methylated HeLa genomic DNA with 4 µl ^{3}H AdoMet, and 8 units of CpG Methylase (M. SssI) for 4 hours at 37°C in 50 µl of 50 mM Tris-HCl (pH 7.8), 1 mM EDTA and 1 mM dithiothreitol incorporated 0.01% of the total radioactivity.

References:

1. Sambrook, J. and Russell, D. (2001) *Molecular Cloning: A Laboratory Manual,* (3rd ed.), (pp. 6.4–6.12). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Herman, J.G. and Baylin, S.B. (1996). U.S. Patent No. 5,786,146. John Hopkins University School of Medicine.
3. Frommer, M., et.al. (1992) PNAS USA 89, 1827–8131.

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CERTIFICATE OF ANALYSIS