CpG Methylated HeLa Genomic DNA

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 Restriction 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.

References:

N4007S

15 µg Lot: 0061604 Exp: 4/18
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.

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-3H] methionine (AdoMet) Incorporation Assay: Incubation of 1 µg of CpG methylated HeLa genomic DNA with 4 µl 3H 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 dihydrothreitol incorporated 0.01% of the total radioactivity.

References: