CpG Methylated Jurkat Genomic DNA

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N4002S 012160418041

N4002S

15 µg **Lot: 0121604** **Exp: 4/18**
100 µg/ml **Store at –20°C**

Description: Human male Jurkat (human acute T-cell leukemia) genomic DNA that was enzymatically methylated with CpG Methylase (M. SssI), suitable as a positive control in the study of CpG dinucleotide methylation.

Source: Jurkat (acute T-cell leukemia) cells were grown to confluency in RPMI 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.

Applications:

- 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.

Quality Control Assays

Bisulfite conversion followed by Methylation-Specific PCR (MSP): 10 µl (1 µg) of CpG Methylated Jurkat 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 Jurkat 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), 1mM 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.

CERTIFICATE OF ANALYSIS

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