5-Aza-dc Treated Jurkat Genomic DNA

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N4003S 003151117111

N4003S

15 µg **Lot: 0031511** **Exp: 11/17**

100 µg/ml **Store at –20°C**

Description: Genomic DNA purified from human male Jurkat (human acute T-cell leukemia) cells that are treated with 5-aza-2´-deoxycytidin(5-Aza-dc), suitable as a negative control in the study of CpG dinucleotide methylation in the genome.

Source: Jurkat (acute T-cell leukemia) cells were grown to 50% confluency in RPMI plus 10% fetal bovine serum and were treated with a 2 µM 5-aza-2´-deoxycytidine for eight days. Genomic DNA was isolated by a standard genomic purification protocol (1), phenol/chloroform extracted and equilibrated to 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA.

Application:

- A negative 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: 1.88

Quality Control Assays

Bisulfite Sequencing: 10 µl (1 µg) of 5-Aza-dc treated Jurkat Genomic DNA and normal Jurkat Genomic DNA were bisulfite converted (3) and eluted in 40 µl of TE buffer. 5 µl were added to a 20 µl PCR reactions containing primers specific to the fully CpG methylated intergenic spacer (IGS) ribosomal DNA (rDNA). 30% of the CpG dinucleotides normally methylated in the control DNA this region were demethylated in the 5-Aza-dc treated Jurkat Genomic DNA as determined from DNA sequenced from the appropriate sized PCR products.

Note: The potent methyltransferase inhibitor (MTI) 5-aza-2´-deoxycytidine (5-Aza-dc) (Decitabine, Dacogen) causes growth arrest, differentiation, and/or apoptosis of many cell types *in vitro* and *in vivo*. The genomic DNA derived from cells treated with this drug exhibit some lower molecular weight smearing when visualized on a 0.8% agarose gel. Significant (up to 70%) genome-wide CpG demethylation was confirmed by bisulfite sequencing of IGS ribosomal DNA (rDNA).

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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