5-Aza-dc Treated Jurkat Genomic DNA

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N4003S 003160418041

N4003S

15 µg **Lot: 0031604** **Exp: 4/18**
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.

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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