**CpG Methylated NIH 3T3 Mouse Genomic DNA**

**N4005S**

<table>
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<tr>
<th>15 µg</th>
<th>Lot: 0061310</th>
<th>Exp: 10/15</th>
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<tr>
<td>100 µg/ml</td>
<td>Store at –20°C</td>
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**Description:** NIH 3T3 (mouse embryonic fibroblast cell line) genomic DNA that was enzymatically methylated with CpG Methylase (M. SssI), suitable as a positive control in the study of CpG dinucleotide methylation.

**Source:** NIH 3T3 (mouse embryonic fibroblasts) 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.

**A 260/280 Ratio:** 1.91

**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 NIH 3T3 Mouse 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.

**References:**

**Incorporation Assay:** Incubation of 1 µg of CpG Methylated NIH 3T3 Mouse 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 dithiothreitol incorporated 0.01% of the total radioactivity.

**References:**

**Procedure:**
- **Inactivation:** 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).

**Quality Assurance:** Purified free of contaminating proteins and RNA.

**A 260/280 Ratio:** 1.91

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

**References:**