Human DNA (cytosine-5) Methyltransferase (Dnmt1)

1-800-632-7799
info@neb.com
www.neb.com

M0230S 032140315031

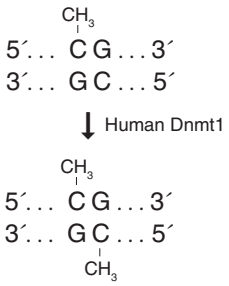
M0230S

BSA RR 37° SAM Yes

50 units **2,000 U/ml** **Lot: 0321403**

RECOMBINANT **Store at –20°C** **Exp: 3/15**

Methylation Site:



Description: Dnmt1 methylates cytosine residues in hemimethylated DNA at 5′...CG...3′ (1,2). Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions (3–5). The bulk of the methylation takes place during DNA replication in the S-S-phase of the cell cycle (6).

Source: Dnmt1 is expressed from human Dnmt1 cDNA using a baculovirus expression system (1,7).

Supplied in: 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 200 mM NaCl, 1 mM dithiothreitol and 50% glycerol. Store at –20°C.

Reagents Supplied with Enzyme:
10X Dnmt1 Reaction Buffer, 100X BSA and 32 mM S-adenosylmethionine (SAM).

Reaction Conditions: 1X Dnmt1 Reaction Buffer, supplemented with 100 µg/ml BSA (supplied) and 160 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X Dnmt1 Reaction Buffer:
50 mM Tris-HCl
1 mM EDTA
1 mM dithiothreitol
5% glycerol
pH 7.8 @ 25°C

Unit Definition: One unit is the amount of enzyme required to catalyze the transfer of 1 pmol of methyl group to poly dI.dC substrate in a total reaction volume of 25 µl in 30 minutes at 37°C.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases.

Storage of SAM: S-adenosylmethionine (SAM) (Sigma Catalog #A7007) is stored at –20°C as a 32 mM solution dissolved in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months. SAM is unstable at (pH 7.5), 37°C (1) and should be replenished in reactions incubated longer than 4 hours. Methylation can be optimized by using fresh SAM.

Heat Inactivation: 65°C for 20 minutes.

Note: For DNA modification and protection applications, M.SssI (NEB #M0226) is preferred because it efficiently methylates both unmethylated and hemimethylated DNA substrates.

References:

1. Pradhan, S. et al. (1999) *J. Biol. Chem.* 274, 33002–33010.
2. Bacolla, A. et al. (1999) *J. Biol. Chem.* 274, 33011–33019.
3. Schmutte, C. et al (1998) *Biol. Chem.* 379, 377–388.
4. Laird, P.W. et al. (1995) *Cell* 81, 197–205.
5. Li, E. et al. (1992) *Cell* 12, 915–926.
6. Leonhardt, H. et al. (1992) *Cell* 71, 865–873.
7. Yen, R.W et al. (1992) *Nucleic Acids Res.* 20, 2287–2291.

CERTIFICATE OF ANALYSIS

Human DNA (cytosine-5) Methyltransferase (Dnmt1)

1-800-632-7799
info@neb.com
www.neb.com

M0230S 032140315031

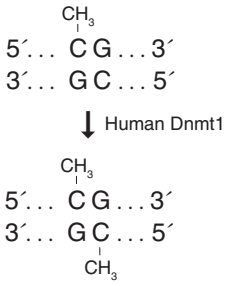
M0230S

BSA RR 37° SAM Yes

50 units 2,000 U/ml Lot: 0321403

RECOMBINANT Store at –20°C Exp: 3/15

Methylation Site:



Description: Dnmt1 methylates cytosine residues in hemimethylated DNA at 5′...CG...3′ (1,2). Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions (3–5). The bulk of the methylation takes place during DNA replication in the S-S-phase of the cell cycle (6).

Source: Dnmt1 is expressed from human Dnmt1 cDNA using a baculovirus expression system (1,7).

Supplied in: 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 200 mM NaCl, 1 mM dithiothreitol and 50% glycerol. Store at –20°C.

Reagents Supplied with Enzyme:
10X Dnmt1 Reaction Buffer, 100X BSA and 32 mM S-adenosylmethionine (SAM).

Reaction Conditions: 1X Dnmt1 Reaction Buffer, supplemented with 100 µg/ml BSA (supplied) and 160 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X Dnmt1 Reaction Buffer:
50 mM Tris-HCl
1 mM EDTA
1 mM dithiothreitol
5% glycerol
pH 7.8 @ 25°C

Unit Definition: One unit is the amount of enzyme required to catalyze the transfer of 1 pmol of methyl group to poly dI.dC substrate in a total reaction volume of 25 µl in 30 minutes at 37°C.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases.

Storage of SAM: S-adenosylmethionine (SAM) (Sigma Catalog #A7007) is stored at –20°C as a 32 mM solution dissolved in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months. SAM is unstable at (pH 7.5), 37°C (1) and should be replenished in reactions incubated longer than 4 hours. Methylation can be optimized by using fresh SAM.

Heat Inactivation: 65°C for 20 minutes.

Note: For DNA modification and protection applications, M.SssI (NEB #M0226) is preferred because it efficiently methylates both unmethylated and hemimethylated DNA substrates.

References:

1. Pradhan, S. et al. (1999) *J. Biol. Chem.* 274, 33002–33010.
2. Bacolla, A. et al. (1999) *J. Biol. Chem.* 274, 33011–33019.
3. Schmutte, C. et al (1998) *Biol. Chem.* 379, 377–388.
4. Laird, P.W. et al. (1995) *Cell* 81, 197–205.
5. Li, E. et al. (1992) *Cell* 12, 915–926.
6. Leonhardt, H. et al. (1992) *Cell* 71, 865–873.
7. Yen, R.W et al. (1992) *Nucleic Acids Res.* 20, 2287–2291.

CERTIFICATE OF ANALYSIS