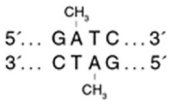
dam Methyltransferase

Catalogue number: MB43401, 500 U

Description

DNA adenine methylase, also known as *dam* Methyltransferase, is an enzyme that adds a methyl group to the adenine of the sequence 5'-GATC-3'. The figure below represents a double stranded sequence after methylation by *dam* Methyltransferase.



dam Methyltransferase belongs to a large group of enzymes unique to prokaryotes and bacteriophages.

Storage conditions

dam Methyltransferase and other kit components should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit of enzyme activity is defined as the amount of enzyme required to protect 1 µg of human genomic DNA in 1 hour at 37 °C in a total reaction volume of 10 µL against cleavage by MboI restriction endonuclease.

Enzyme concentration: 8 U/µL

Inactivation

dam Methyltransferase is heat inactivated by incubation at 65°C for 20 min.

System components and Reaction conditions

dam Methyltransferase is provided with a dedicated and highly optimized NZYTech 10x reaction buffer. In addition, a 400x solution of S-adenosylmethionine (SAM; 32 mM) is provided. The enzyme displays an optimum temperature of 37 °C.

Standard protocol

The following standard protocol serves as a general guideline for the methylation of genomic DNA. Preferably the enzyme should be added last.

1. Prepare a fresh 1/20 dilution of the provided SAM (32 mM stock solution) to 1.6 mM (e.g., to prepare 20 µL of diluted solution, add 1 µL of SAM in 19 µL of Nuclease-free Water).

2. Prepare the following 50 µL reaction:

Component	Volume
Substrate DNA (*)	1 µg
dam Methyltransferase reaction buffer (10x)	5 µL
Diluted SAM (1.6 mM)	5 µL
dam Methyltransferase	1 µL (¥)
Nuclease-free H_2O (Cat. No. MB11101)	up to 50 µL

(*) *Besides genomic DNA, other types of DNA can be used as substrate, such as DNA plasmids and purified PCR products.*
(¥) *4-25 units methyltransferase/µg of DNA is recommended.*

3. Gently mix and pulse.

4. Incubate at 37 °C for 1 hour. The incubation time can be increased to 4 hours. Overnight incubations do not give significant increases in methylation.

5. Stop the reaction by heating at 65°C for 20 minutes.

6. To obtain a highly pure product, perform a column purification step using NZYGelpure kit (NZYTech, Cat. No. MB011).

Quality Control Assays

Purity

dam Methyltransferase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with *dam* Methyltransferase for 14-16 hours at 37 °C. Following incubation, the nucleic acid is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

dam Methyltransferase is assayed in a typical methylation reaction using human genomic DNA as substrate. The extent of DNA protection is determined by digestion with MboI restriction enzyme as judged through agarose gel electrophoresis.

V2101

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases contamination	Pass
Functional assay	Pass

Approved by:

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