Methyl-directed DNA Endonuclease

Mte I

E554

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2500 u **Lot: 1**
10 000 u/ml **Store at -20°C**

Recognition sequence

5'-G(5mC)G(5mC)^NG(5mC)G(5mC)-3'
3'-(5mC)G(5mC)GN^(5mC)G(5mC)G-5'

Sourse: ***Microbacterium testaceum 17B***
Substrate specificity:

The enzyme cleaves C5-methylated DNA and doesn't cut unmodified DNA.

Supplied in:
10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 200 µg/ml BSA; 50% glycerol.

Reaction Conditions:
1×SEBuffer **W**. Incubate at **55°C**.

1×SEBuffer W (pH 8.5 @ 25ºC)
Warranty period for the enzyme storage at-20˚C is two years from the date of the last assay indicated on the enzyme vial.

10 mM Tris-HCl 150 mM NaCl
10 mM $MgCl_2$; 1 mM DTT

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze completely1 µg of linearized plasmid pHspAI10/DriI+M.Fsp4HI in 1 hour at 55°C in a total reaction volume of 50 µl.

pHspAI10/DriI+M.Fsp4HI is a plasmid pHspAI10, which is linearized with DriI, and, additionally, modified with Fsp4HI DNA methyltransferase pHspAI10 carries a gene of HspAI DNA methyltransferase, that modifies the sequence . 5`-GCGC-3`, producing 5`-G(5mC)GC-3`.
M.Fsp4HI modifies the sequence 5`-GCNGC-3`, producing 5`-G(5mC)NGC-3`.
A substrate pHspAI10/DriI+M.Fsp4HI includes one site 5`-G(5mC)G(5mC)NG(5mC)G(5mC)-3`/3`-(5mC)G(5mC)GN(5mC)G(5mC)G-5`, which is MteI canonical site [1]. The enzyme activity depends on a number and positions of methylated nucleotides in the recognition sequence.
For example, MteI cuts the recognition site with six 5-methylcytosines, but the enzyme activity is reduced for more that one order [1].

Warranty period for the enzyme storage at-20˚C is two years from the date of the last assay indicated on the enzyme vial.

Enzyme Properties

Activity in SEBuffers:

SEBuffer B	25-50%
SEBuffer G	75-100%
SEBuffer O	75-100%
SEBuffer W	100%
SEBuffer Y	50-75%
SEBuffer ROSE	100%

When using a buffer other than the optimal (suppied) SEBuffer, it may be necessary to add more enzyme to achieve a complete digestion.

Quality Control Assays

16-Hour Incubation:
No detectable degradation of 1µg of Lambda DNA was observed after incubation with 10 units of enzyme for 16 hours at 55°C in a total reaction volume of 50 µl.

Oligonucleotide Assay:
No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 10 units of enzyme for 3 hours.

Reagents Supplied with Enzyme: 10×SEBuffer W

Heat Inactivation: No (80°C for 20 minutes)

References:

1.***V.A. Chernukhin, E.V. Kileva, V.A. Sokolova., D.A. Gonchar, L.N. Golikova, V.S. Dedkov, N.A. Mikhnenkova, S.Kh. Degtyarev***
A new methyl-directed site-specific DNA endonuclease MteI cleaves nine nucleotides sequence
5'-G(5mC)G(5mC)^NG(5mC)GC-3'/3'-CG(5mC)GN^(5mC)G(5mC)G-5'
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CERTIFICATE OF ANALYSIS