Methyl-directed DNA Endonuclease

BlsI

E533

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100 u **Lot: 9**
5000 u/ml **Store at -20°C**

Recognition sequence with at least two 5mC:

5'… PuPyN↓PuPy …3'
3'… PyPu↑NPyPu …5'

except 5'-A(5mC)NGT-3'
3'-TGN(5mC)A-5'

Sourse: ***Bacillus simplex 23***

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

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

Reaction Conditions:
1×SEBuffer **W**. Incubate at **30°C**.

1×SEBuffer W (pH 8.5 @ 25°C)
10 mM Tris-HCl 100 mM NaCl
10 mM $MgCl_2$ 1 mM DTT

Substrate specificity:
BlsI cleaves DNA sequence 5'- PuPyNPuPy-3'/3'-PyPuNPyPu-5', if at least two 5-methylcytosines are present in the recognition site (N isn't considering). The enzyme activity varies for different sites and depends on number and position of methylated cytosines in the recognition sequence.
BlsI doesn't cleave DNA sequence 5'-A(5mC)NGT-3'/3'-TGN(5mC)A-5'.

Optimal recognition site:

5`-G(5mC)NGC-3`/3`-(5mC)GN(5mC)G-5`

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze at least one of three canonical sites: 5`-G(5mC)NG(5mC)-3`/3`-CGN(5mC)G-5` in 1 μg of linearized plasmid pFsp4HI3 in 1 hour at 30°C in a total reaction volume of 50 μl. Concentrated enzymes are diluted to approximately 1000 units/ml with the buffer [10 mM Tris-HCl (pH 7.6); 50 mM KCl; 0.1 mM EDTA; 1 mM DTT; 200 μg/ml BSA; 50% glycerol] before the activity determination.

DNA pFsp4HI3/DriI is a linearized plasmid pFsp4HI3, which carries a gene of DNA-methyltransferase M.Fsp4HI and includes three canonical sites:

5`-G(5mC)NG(5mC)-3`/3`-CGN(5mC)G-5` [2].

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 30°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 5 units of enzyme for 3 hours.

Enzyme Properties

Activity in SEBuffers:

SEBuffer B 10-25%
SEBuffer G 10-25%
SEBuffer O 50-75%
SEBuffer W 100%
SEBuffer Y 75-100%
SEBuffer ROSE 50%

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

Reagents Supplied with Enzyme: 10×SEBuffer W

Heat Inactivation: Yes (65°C for 20 minutes)

References:

1. **Chernukhin V.A., Tomilova J.E., Chmuzh E.V., Sokolova O.O., Dedkov V.S., Degtyarev S.Kh.** Bacterial strain Bacillus simplex - producer of BlsI site specific endonuclease. // Russian Federation patent RU 2322494 C1 (2006).

2. **Chmuzh E.V., Kashirina Yu.G., Tomilova Yu.E., Chernukhin V.A., Okhapkina S.S., Gonchar D. A., Dedkov V.S., Abdurashitov M. A., Degtyarev S. Kh.** Gene cloning, comparative analysis of the protein structures from Fsp4HI restriction-modification system and biochemical characterization of the recombinant DNA methyltransferase M.Fsp4HI. // Molecular Biology, V.41, No 1, p. 43-50 (2007)

CERTIFICATE OF ANALYSIS