

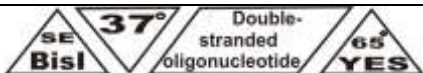
Methyl-directed DNA
Endonuclease



SibEnzyme
SibEnzyme Ltd., Russia
Ph: +7 383 333 4991
Fax :+7 383 333 6853
info@sibenzyme.com
www.sibenzyme.com

Bis I

E485



40 u

Lot: 9

1000 u/ml

Store at -20° C

Recognition Sequence:

5'...G(5mC)↓N G C...3'

3'...C G N↑(5mC)G...5'

Source: *Bacillus subtilis* T30

The enzyme cleaves only methylated DNA

Supplied in:

10 mM Tris-HCl (pH 7.5); 100 mM NaCl;
0,1 mM EDTA; 7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:

1×SEBuffer Bis I

Incubate at **37°C**.

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

1×SEBuffer BisI (pH 9.0 @ 25°C)

10 mM Tris-HCl 150 mM KCl

10 mM MgCl₂ 1 mM DTT

Unit Definition:

One unit is defined as the amount of enzyme required to cleave

1 pmol of the double-stranded oligonucleotide with the following structure

5' GCTTGACTTTAG(5mC)GGCATTGATTCTCACCACG 3'

3' CGAACATGAAATCGC(5mC)GTAAC TAAGAGTGGTGC 5'

in 1 hour at 37°C in a total reaction volume of 20 µl.

Reagents Supplied with Enzyme:

10×SEBuffer Bis I

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

Quality Control Assays

16-Hour Incubation: No nonspecific activity was detected after incubation of 1 µg of pFsp4HI1 DNA (BamHI digest) with 1 unit of BisI for 16 hours at 37°C. The pFsp4HI1 plasmid carries a gene for Fsp4HI DNA-methyltransferase, which modifies DNA forming 5'-G(5mC)NGC-3'/3'-CGN(5mC)G-5'.

Oligonucleotide Assay:

No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 1 units of enzyme for 3 hours.

Enzyme Properties

Activity in SEBuffers:

SEBuffer B 10-25%

SEBuffer G 25-50%

SEBuffer O 50-75%

SEBuffer W 75-100%

SEBuffer Y 50--75%

SEBuffer ROSE 30%

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

CERTIFICATE OF ANALYSIS