Methyl-directed DNA Endonuclease

Gla I

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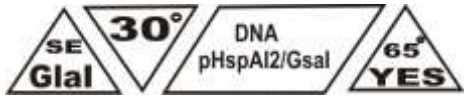

500 u **Lot: 32**
8000 u/ml **Store at -20°C**

Recognition Sequence:

5'…Pu(5mC)↓GPy…3'
3'…PyG↑(5mC)Pu…5'

Sourse: ***Glacial ice bacterium GL29***

The enzyme cleaves C5-methylated DNA and does not cut unmodified DNA and DNA with N4-methylcytosines [1].

Warranty period for the enzyme storage at-20˚C is one year 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; 0.05% Triton X-100; 100 μg/ml BSA;7 mM 2-mercaptoethanol; 50% glycerol.

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

1×SEBuffer GlaI (pH 8.5 @ 25ºC)
10 mM Tris-HCl, 10 mM NaCl, 5 mM $MgCl_2$,1 mM 2-mercaptoethanol.

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

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyse completely a unique 5`-G(5mC)G(5mC)-3`/3`-(5mC)G(5mC)G-5` site in 1 μg of pHspAI2 plasmid DNA, which is linearized with GsaI, in 1 hour at 30ºC 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 pHspAI2/GsaI is a linearized plasmid pHspAI2, which carries a gene of DNA-methyltransferase M.HspAI (recognition sequence 5'-GCGC-3') and includes a unique GlaI recognition site 5'-G(5mC)G(5mC)-3'/3'-(5mC)G(5mC)G-5' [2].

Substrate specifity [3]

The enzyme activity depends on number and position of methylated nucleotides in the recognition sequence:
Optimal substrate (100% activity): 5`-G(5mC)G(mC)-3`/3`-(m5C)G(m5C)G-5`.
Good substrates (> 25% activity): 5`-R(5mC)G(5mC)-3`/3`-YG(5mC)G-5/ 5`-A(5mC)GT-3`/3`-TG(5mC)A-5`.
Medium substrates (> 6% activity): 5`-G(5mC)R(5mC)-3`/3`-(5mC)GYG-5` / 5`-G(5mC)GT-3`/3`-CG(5mC)A-5`.
Bad substrates (6% activity): 5`-G(5mC)GC-3`/3`-CG(5mC)G-5`.

Quality Control Assays

16-Hour Incubation:
No detectable degradation of 1 μg of Lambda DNA was observed after incubation with 8 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 8 units of enzyme for 3 hours.

Activity in SEBuffers:
SEBuffer B 75-100%
SEBuffer G 75-100%
SEBuffer O 75-100%
SEBuffer W 75-100%
SEBuffer Y 75-100%
SEBuffer ROSE 100%

Reagents Supplied with Enzyme: 10×SEBuffer GlaI, DNA pHspAI2/GsaI.

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

References:

1.Chernukhin V.A., Nayakshina T.N., Tomilova J.E., Mezentseva N.V., Dedkov V.S., Degtyarev S.Kh. Bacterial strain Glacial ice bacterium I - producer of GlaI restriction endonuclease. // Russian Federation patent RU 2287012 C1 (2006).
2.Chernukhin V.A., Najakshina T.N., Abdurashitov M.A., Tomilova J.E., Mezentzeva N.V., Dedkov V.S., Mikhnenkova N.A., Gonchar D.A., Degtyarev S. Kh A novel restriction endonuclease GlaI recognizes methylated sequence 5'-G(5mC)^GC-3'.// Biotechnologia V 4. P. 31-35(2006)
3.Tarasova G. V., Nayakshina T. N., Degtyarev S. Kh. Substrate specificity of new methyl-directed DNA endonuclease GlaI . // BMC Molecular Biology 2008, 9:7

CERTIFICATE OF ANALYSIS