Restriction Endonuclease

SibEnzyme®

Gla I

Recognition Sequence: Pu(5mC)↓GPy
PyG↑(5mC)Pu

L

E494

5,000 units

50,000 u/ml

Lot:

Exp:

Store at -20°C

SE-Buffers	B	G	O	W	Y	ROSE
%Activity	75-100	75-100	25-50	25-50	100	100

30°C | 65°C | Y | pHspAI2/GsaI | RR

For more details scen the code

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CERTIFICATE OF ANALYSIS

Source: *An E.coli strain that carries the cloned Gla I gene from Glacial ice bacterium Gl29.*

Supplied in:
10 mM Tris-HCl (pH 7.6), 250 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100, 7 mM 2-mercaptoethanol, 50 µg/ml BSA, 50% glycerol.

Reaction Conditions:
1X SE-Buffer Y. Incubate at 30°C.

1X SE-Buffer Y (pH 7.9 @ 25° C):
33 mM Tris-Ac 66 mM KAc
10 mM MgAc 1 mM DTT

Heat Inactivation:
Enzyme is inactivated by incubation at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to digest 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 30° C in a total reaction volume of 50 µl. 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`.

Quality Control Assays
16-Hour Incubation: No detectable degradation of 1µg of Lambda DNA was observed after incubation with 100 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-stranded and double-stranded oligonucleotide was observed after incubation with 50 units of restriction endonuclease for 3 hours.

Enzyme Properties:
When using a buffer other than the optimal (Supplied) SE-Buffer, it may be necessary to add more enzymes to achieve complete digestion.

Substrate specificity:
The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA and DNA with N4-methylcytosines
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`-(5mC)G(5mC)G-5`
Good substrates (> 25% activity)
5`-R(5mC)G(5mC)-3`/3`-YG(5mC)G-5`
5`-R(5mC)GY-3`/3`-YG(5mC)R-5`
Medium substrates (> 6% activity)
5`-G(5mC)R(5mC)-3`/3`-(5mC)GYG-5`
5`-R(5mC)GY-3`/3`-YG(5mC)R-5`
Bad substrates (6% activity)
5`-G(5mC) GC-3`/3`-CG(5mC)G-5`

Reagents Supplied with Enzyme:
10X SE Buffer Y, DNA pHspAI2/GsaI.