

Methyl-directed DNA  
Endonuclease



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**Glu I**

**E520**



250  $\mu$

Lot: 8

1000  $\mu$ /ml

Store at -20° C

Recognition Sequence:

5'... G(5mC)↓NG(5mC)...3'  
3'... (5mC)GN↑(5mC)G...5'

Source: *Glacial ice bacterium* GL24

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

Supplied in:

10 mM KH<sub>2</sub>PO<sub>4</sub> (pH 7.45); 200 mM NaCl; 0,1 mM EDTA; 200  $\mu$ g/ml BSA;  
7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:

1×SEBuffer Y

Incubate at 37°C.

1×SEBuffer Y (pH 7.9 @ 25°C)

33 mM Tris-Ac 66 mM MgAc

66 mM KAc 1 mM DTT

Substrate specificity:

The enzyme activity depends on number and position of methylated cytosines.

Optimal recognition site:

5'-G(5mC)↑NG(5mC)-3'/3'-(5mC)GN↓(5mC)G-5'

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze completely a unique site 5'-G(5mC)NG(5mC)-3'/3'-(5mC)GN(5mC)G-5' in 1  $\mu$ g of linearized plasmid pFsp4HI3/Dril in 1 hour at 37°C in a total reaction volume of 50  $\mu$ l.

DNA pFsp4HI3/Dril is a linearized plasmid pFsp4HI3, which carries a gene of DNA-methyltransferase M.Fsp4HI and includes a unique GluI site:  
5'-G(5mC)NG(5mC)-3'/3'-(5mC)GN(5mC)G-5' [2].

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

Quality Control Assays

16-Hour Incubation:

No detectable degradation of 1  $\mu$ g of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 37°C in a total reaction volume of 50  $\mu$ l.

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 75-100%

SEBuffer G 75-100%

SEBuffer O 25-50%

SEBuffer W 50-75%

SEBuffer Y 100%

SEBuffer ROSE 100%

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

Reagents Supplied with Enzyme: 10×SEBuffer Y

Heat Inactivation: Yes (80° C for 20 minutes)

References:

1. Chernukhin V.A., Chmuzh E.V., Tomilova J.E., Nayakshina T.N., Dedkov V.S., Degtyarev S.Kh. Bacterial strain Glacial ice bacterium - producer of GluI site specific endonuclease. // Russian Federation patent RU 2322492 C1 (2006).

2. Chernukhin V.A., Chmuzh E.V., Tomilova Yu.E., Nayakshina T.N., Gonchar D.A., Dedkov V.S., Degtyarev S.Kh. A novel site-specific endonuclease GluI recognizes methylated DNA sequence 5'-G(5mC)NG(5mC)-3'/3'-(5mC)GN(5mC)G-5'.// Bulletin of biotechnology and physico-chemical biology named by Yu.A.Ovchinnikov (Moscow), V.3, No.2, p.13-17 (2007).

**CERTIFICATE OF ANALYSIS**