Methyl-directed DNA Endonuclease

PkrI

E579

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50 u **Lot: 1**
1000 u/ml **Store at -20°C**

Recognition sequence

5'… G(5mC)N↓G(5mC)…3'
3'… (5mC)G↑N(5mC)G …5'

Sourse: ***Planomicrobium koreense*** **78k**

Substrate specificity:

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

PkrI cleaves DNA sequence 5`- GCNGC-3`/3`-CGNCG-5', if **at least three** 5-methylcytosines are present in the recognition site (N isn`t considering) [1].

Optimal recognition sites (100% activity):

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

Supplied in:
20 mM Tris-HCl (pH 7.4); 200 mM NaCl; 0,1 mM EDTA;
7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:
1×SEBuffer **Y**. Incubate at **37°C**.

1×SEBuffer Y (pH 7.9 @ 25ºC)

33 mM Tris-Ac 66 mM KAc
10 mM MgAc 1 mM DTT

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 37°C in a total reaction volume of 50 µl.
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 2 units of enzyme for 16 hours at 37°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 2 units of enzyme for 3 hours.

Enzyme Properties

Activity in SEBuffers:

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

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

References:

1. . ***V.A. Chernukhin, T.N. Nayakshina, D.A. Gonchar, Ju.E. Tomilova, M.V. Tarasova, V.S. Dedkov, N.A. Mikhnenkova, S.Kh. Degtyarev.*** A new site-specific methyl-directed DNA endonuclease PkrI recognizes and cuts methylated DNA sequence 5'-GCN^GC-3'/3'-CG^NCG-5' carrying at least three 5-methylcytosines. // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.7, No.3, pp. 35-42, 2011 (Rus.).

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