

FspEI



1-800-632-7799
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www.neb.com



R0662S 001120614061

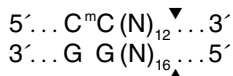
R0662S



200 units **4,000 U/ml** **Lot: 0011206**

RECOMBINANT **Store at -20°C** **Exp: 6/14**

Recognition Site:

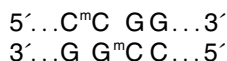


Description: FspEI, an EpiMark® validated product, is a modification-dependent endonuclease which recognizes C^mC sites and generates a double-stranded DNA break on the 3' side of the modified cytosine at N₁₂/N₁₆. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC) (1).

This enzyme is provided with an Enzyme Activator Solution which may be used for efficient digestion by FspEI.

The most common epigenetic modifications found in eukaryotic organisms are methylation marks at CpG or CHG sites. A subset of these modified sites are recognized and cleaved by FspEI.

At fully methylated CpG sites:



or CHG sites:



H = A or C or T (not G)
D = A or G or T (not C)

FspEI recognizes each hemi-methylated site individually and cleaves bidirectionally to generate 32 base or 31 base fragments, respectively. These fragments contain the central methylated site and have 4-base 5' overhangs at each end. FspEI does not cleave unmodified DNA.

Source: An *E. coli* strain that carries the synthetic FspEI gene from *Frankia* species EAN1pec

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 4, 30X Enzyme Activator Solution, 100X BSA

Reaction Conditions: 1X NEBuffer 4, supplemented with Enzyme Activator Solution and BSA. Incubate at 37°C.

1X NEBuffer 4:

50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBR322 (dcm⁺) DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Assurance: The EpiMark suite of products have been validated for use in an epigenetic application.

Usage Notes: Use of excess enzyme inhibits cleavage. Optimization of the amount of enzyme needed for complete digestion may be required for each substrate DNA. Excess of enzyme or prolonged digestion time in the presence of Enzyme Activator Solution may cause star activity.

Protocol for Genomic DNA Digestion:

- Set up the following reaction in a sterile micro-centrifuge tube (it is important to add FspEI last):

DNA (0.5 to 1 µg)	1–5 µl
10X NEBuffer 4	3 µl
BSA	1 µl
30X Enzyme Activator Solution	1 µl
FspEI	0.5–1 µl (2 to 4 units)
Nuclease-free Water	to 30 µl

- Incubate at 37°C for 4–8 hours. *(see other side)*
CERTIFICATE OF ANALYSIS

FspEI



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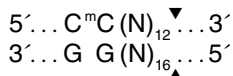
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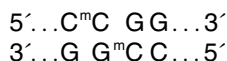


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Nuclease-free Water	to 30 µl

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CERTIFICATE OF ANALYSIS

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of unmethylated substrate [pBR322 (dcm-)] and 20 units of FspEI incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of 20 units of FspEI with 1 µg of a mixture of single and double-stranded ³H *E. coli* DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

RNase Assay: Incubation of a 10 µl reaction containing 20 units of FspEI with 40 ng of RNA transcript for overnight at 37°C resulted in no detectable degradation of the RNA as determined by agarose gel electrophoresis.

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Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 NR
NEBuffer 2 NR
NEBuffer 3 NR
NEBuffer 4 **100%**

Survival in a Reaction: A minimum of 0.16 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes

References

1. Zheng, Y. et al. (2010) *Nucl. Acids Res.* doi:10, 1093/nar/gkq327.
2. U.S. Publication No. 2010-0167942

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