Thermostable FEN1

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

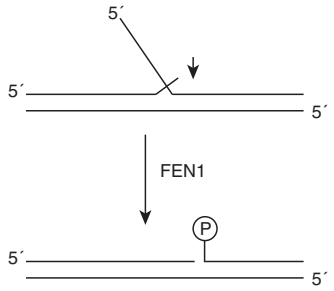
M0645S 001160918091

M0645S

1,600 units **32,000 U/ml** **Lot: 0011609**
RECOMBINANT **Store at –20°** **Exp: 9/18**

Description: Thermostable Flap Endonuclease 1, FEN1, catalyzes the cleavage of 5´ DNA flaps from branched double stranded DNA substrates, creating a 5´ phosphate terminus. FEN1 products can be ligated by DNA ligase to create double stranded DNA. *In vivo*, FEN1 is an essential component of the Okazaki fragment maturation pathway, and also plays a role in base excision repair.

Reaction Schematic Thermostable FEN1 recognizes and cleaves 5´ DNA flaps generating a ligatable 5´ phosphate terminus.



Source: An *E. coli* strain, which carries the cloned Thermococcus 9°N™ FEN1 gene.

Applications:

- Cleavage of flap DNA structure

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mm DTT, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X ThermoPol® Reaction Buffer.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 65°C.

1X ThermoPol Reaction Buffer:
10 mM KCl
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
2 mM $MgSO_4$
0.1% Triton X-100
pH 8.8 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of 5´ flap containing oligonucleotide substrate in a total reaction volume of 10 µl for 10 min at 65°C.

Diluent Compatibility: Diluent D
100 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

Unit Assay Conditions: 1X ThermoPol Reaction Buffer containing 0.2 pmol of fluorescently labeled oligonucleotide in a total reaction volume of 10 µl.

Quality Control Assays

Physical Purity (SDS PAGE): Thermostable FEN1 is > 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

Non-specific DNase Activity (16 hour): A 50 µl reaction in Thermopol Buffer containing 1 µg of λ-HindIII DNA and 320 units of Thermostable FEN1 incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by gel electrophoresis.

Exonuclease Activity (Radioactivity Release): A 50 µl reaction in Thermopol Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and 160 units of Thermostable FEN1 incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

CERTIFICATE OF ANALYSIS

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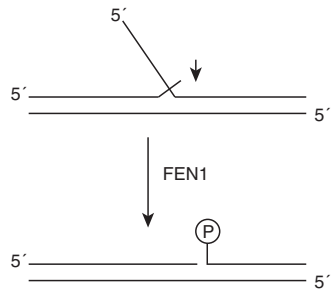

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CERTIFICATE OF ANALYSIS

Endonuclease Activity (Nicking): A 50 µl reaction in Thermopol Buffer containing 1 µg of ϕX174 RF I DNA and a minimum of 160 units of Thermostable FEN1 incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

RNase Activity (Extended Digestion): A 10 µl reaction in NEBuffer 4 containing 40 ng of F-300 RNA transcript and a minimum of 1 µl of Thermostable FEN1 was incubated at 37°C. After 16 hrs, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Heat Inactivation: no

References:

1. Balakrishnon, L. et al. (2013) *Annu. Rev. Biochem.* 82, 119–138.
2. Greenough, L. et al. (2015) *J. Biol. Chem.* 20, 12514–12522.
3. Greenough, L. et al. (2015) *Nucleic Acids Res.* doi:10.1093/nar/gkv899.

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