TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures.

Human FEN-1 (Flap Endonuclease)

Catalog #: 4120-100-EB

Contents:		**Size:**
4120-100-01 Human FEN-1		100 Units
4120-100-02 10X BSA Additive		500 µl
3900-500-12 10X REC™ Reaction Buffer 12		1 ml

Description: FEN-1 is a 50 kDa endonuclease/exonuclease that functions in mammalian replication and repair. The enzyme shows similarity to the yeast Rad2 and Rad13 genes. FEN-1 was identified as a necessary component of Okazaki fragment processing and functions on a number of branched DNA structures. Recently, it was shown that FEN-1 specifically associates with PCNA and this binding stimulates FEN-1 up to 50 fold.

Source: Purified from *E. coli* containing a recombinant plasmid encoding the human FEN-1 protein.

Unit Definition: One Unit cleaves 1 pmole of a ^{32}P-labeled oligonucleotide probe within an oligonucleotide flap complex in one hour at 30˚C.

Specificity: FEN-1 catalyzes the nucleotide excision of DNA branch structures formed during replication of the lagging strand of DNA. The branch structures are formed due to the frequent mismatches that occur at the 5'-end of Okazaki fragments. FEN-1 is also involved in the endonucleolytic cleavage of branch structures that are formed during DNA double strand break end-joining. FEN-1 cleaves DNA flap structures one nucleotide distal to the elbow in the single-stranded region or one nucleotide proximal to the elbow in the double-stranded region, creating two possible cleavage products (see figure overleaf). FEN-1 also has a 5'-3' exonuclease activity specific for recessed 5' ends. This exonuclease activity may be involved in the removal of initiator RNA of mammalian Okazaki fragments.

Assay Conditions: The flap substrate is prepared by hybridizing 50 pmole Flap Oligonucleotide (Cat# 3862-100-01) labeled with ^{32}P, 100 pmole Bridge Oligonucleotide (Cat# 3862-100-02), and 100 pmol Adjacent Oligonucleotide (Cat# 3862-100-03) in 20 mM Tris (pH 7.4),15 mM NaCl in a 50 µl volume at 99 ˚C for 10 minutes and then cooling slowly to 4˚C. 1X REC™ Reaction Buffer 12, 1X BSA Additive (0.1 mg/ml BSA, 5 % glycerol), 4 µl of the above ^{32}P flap substrate, and serial dilutions of enzyme in a reaction volume of 10 µl are incubated for 1 hour at 30˚C. For analysis, 2 µl of 5X REC™ Loading Buffer (Cat# 4018-250: 20 mM EDTA, 20% Ficoll, amd 0.2% bromophenol blue) are added, and cleavage products are resolved by 20% denaturing polyacrylamide gel electrophoresis. The bands are cut out and radioactivity counted to quantify the cleavage products.

Storage Conditions: Store at -20˚C in a manual defrost freezer.

Storage Buffer: 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM DTT, 0.1 mg/ml BSA, and 50% (v/v) glycerol.

References:
1. Harrington, J.J., and M.R. Lieber. 1994. The characterization of a mammalian DNA structure-specific endonuclease. *EMBO Journal* **13**:1235-1246.
2. Wu, X., J. Li, X. Li, C.-L. Hsieh, P.M.J. Burgers, and M.R. Lieber. 1996. Processing of branched DNA intermediates by a complex of human FEN-1 and PCNA. *Nucleic Acids Res.* **24**:2036-2043.

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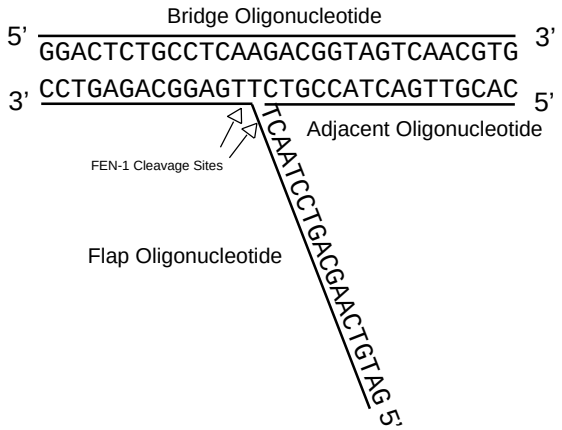
TREVIGEN®

8405 Helgerman Court, Gaithersburg, MD 20877 USA

Voice: 1-800-TREVIGEN (1-800-873-8443) • 301-216-2800

Fax: 301-216-2801 • e-mail: info@trevigen.com • www.trevigen.com



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