Endonuclease IV

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M0304S 004151016101

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1,000 units	**10,000 U/ml**	**Lot: 0041510**
RECOMBINANT	**Store at –20°C**	**Exp: 10/16**

Description: Endonuclease IV can act on a variety of oxidative damage in DNA (1). The enzyme is apurinic/apyrimidinic (AP) endonuclease that will hydrolyse intact AP sites in DNA. AP sites are cleaved at the first phosphodiester bond that is 5´ to the lesion leaving a hydroxyl group at the 3´ terminus and a deoxyribose 5´-phosphate at the 5´ terminus. The enzyme also has a 3´ -diesterease activity and can release phosphoglycoaldehyde, intact deoxyribose 5-phosphate and phosphate from the 3´ end of DNA.

Source: An *E. coli* strain which carries the cloned Endo IV gene

Applications:

- Single cell gel electrophoresis (Comet assay) (2,3,4)
- Alkaline elution (5)
- Alkaline unwinding (6)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl,
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 @ 25°C

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

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C.

*An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Unit Assay Conditions: 1X NEBuffer 3 containing 10 pmol of flourescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Quality Assurance: Purified free of contaminating exonucleases and endonucleases.

Recommended Dilution for the Comet Assay: 1.10^{4} to $1:10^{5}$ (2,3,4,7). A detailed protocol can be found at www.neb.com.

Heat Inactivation: 85°C for 20 minutes.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stability.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 100 units of Endonuclease IV incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

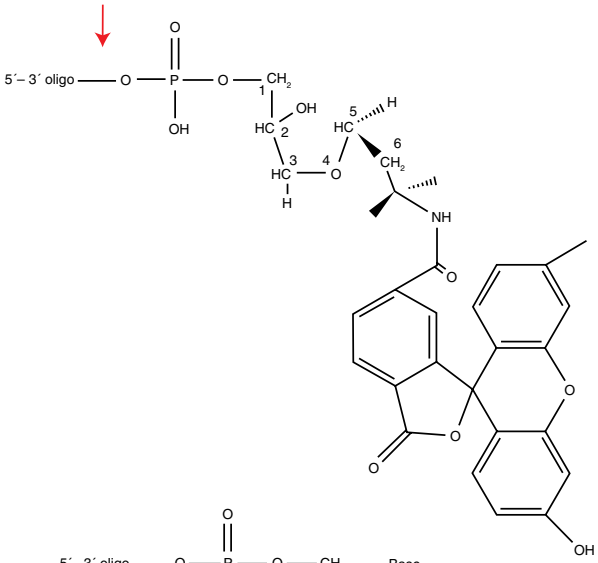
Exonuclease Activity: Incubation of a 50 µl reaction containing 10 units of Endonuclease IV with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 100 units of Endonuclease IV with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

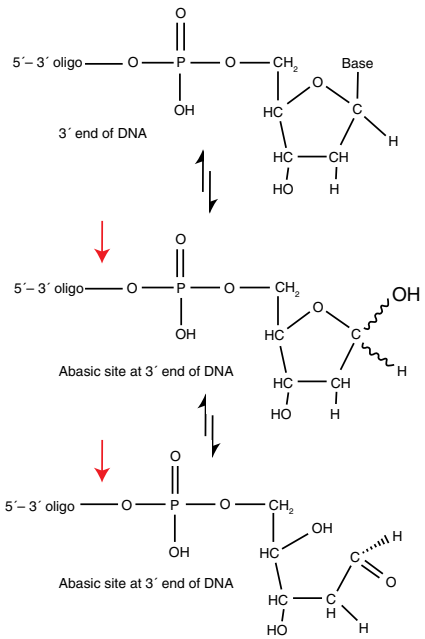
References:

1. Levin, J. et al. (1988) *J. Gen. Physiol.* 33, 349–362.
2. Singh, N. et al. (1961) *Experimental Cell Reseach* 175, 184–191.
3. Collins, A. et al. (1993) *Carcinogenesis* 14, 1733–1735.
4. Collins, A. et al. (1996) *Environmental Health Perspectives* 104, 465–469.
5. Pflaum, M. et al. (1998) *Free Rad. Res.* 29, 585–594.
6. Hartwig, A. et al. (1996) *Toxicology Letters* 88, 85–90.
7. Marks, K., New England Biolabs, Inc. unpublished observations.
8. Marks, K. and Landry D., New England Biolabs, Inc., unpublished observations.

Figure 1: *The 6-carbon phosphodiester spacer used in the 3´ fluorescent label of synthetic oligonucleotides is structurally similar to an abasic site. Removal of the fluorescent label from the 3´ end of oligonucleotide substrates by Endo IV has been observed (8).*



The structure of a 3´ fluorescently labeled oligonucleotide. Red arrow indicates where Endo IV cleaves.



Depurination/depyrmidination at the 3´ end of an oligonucleotide generates an abasic site. The red arrows indicate where Endo IV may cleave.

CERTIFICATE OF ANALYSIS

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.