Depurination/depyrimidination at the 3´ end of DNA.

**Endonuclease IV**

**M0304S**

1,000 units 10,000 U/ml Lot: 0041211

RECOMBINANT Store at –20°C Exp: 11/13

**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´-diesterase 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.

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

1X NEBuffer 3:
- 100 mM NaCl,
- 50 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM diithothreitol
- 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.

**Reagents Supplied with Enzyme:**
- 10X NEBuffer 3.

**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 fluorescently 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:** 1.10^10:1.10^7 (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 a µg of a mixture of single and double-stranded [32P] E. coli DNA (10^6 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:**
7. Marks, K., 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).