Endonuclease IV

Description: Endonuclease IV can act on a variety of oxidative damage in DNA (1). The enzyme is apurinic/apyrimidinic (AP) endonuclease that will hydrolyze 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)

Reagents Supplied with Enzyme:
- 10X NEBuffer 3.

Reaction 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 exonuclease 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 Coo massie 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 [3H] 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 Marks, K., New England Biolabs, Inc. unpublished observations.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).