Human Apurinic/Apyrimidinic Endonuclease (APE)
Catalog Number: EN004

Specifications and Use

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<td>10X DDR Buffer 2 (100 mM HEPES-KOH (pH 7.4), 1 M KCl, 100 mM MgCl₂)</td>
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Description

♦ Human APE (also known as Ref-1) is the apurinic/apyrimidinic (AP) endonuclease required for efficient DNA base excision repair (BER). Following the removal of a damaged base by a DNA glycosylase, APE cleaves the AP site to allow resynthesis and ligation to complete repair. In addition, APE/Ref-1 acts as a factor that regulates the redox state of multiple transcription factors, including c-Jun, c-Fos, NF-κB, and p53.

Source

♦ A cDNA corresponding to amino acid residues 2-318 of human APE was fused to a sequence encoding a 6X histidine epitope tag at the N-terminus. The recombinant fusion protein was expressed in E. coli.

Unit Definition

♦ One unit is the amount of enzyme required to cleave the tetrahydrofuran (THF) synthetic AP Site Oligonucleotide (Cat. # 3854-100-OL) indicated below at the rate of 1 pmole/hour at 37° C. Comparable results were observed when the AP Site Oligonucleotide was used (Cat. # 3851-100-OL).

♦ Sequence:

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5’ CCTGCCCTG_{318} GCAGCTG TGGG 3’
3’ GGACGGGAC ACGTCGACACCC
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Specificity

♦ Human APE catalyzes the cleavage of the phosphodiester bond either 3’ or 5’ to an AP site.

Assay Conditions

♦ Serial dilutions of human APE are incubated for 1 hour at 37° C in a 20 µL reaction containing 1X DDR Buffer 2 and 4 pmole THF-AP Site Oligonucleotide (Cat. # 3854-100-OL) labeled with ³²P and annealed with 4 pmole Oligo Complement B (Cat. # 3854-100-OL). Following the addition of 10 µL of 3X Alkali Loading Buffer (300 mM NaOH, 97% formamide, 0.2% bromophenol blue), samples are heated to 95° C for 10 minutes and fast cooled on ice. Cleavage products are resolved by 20% polyacrylamide gel electrophoresis, excised from the gel, and quantified by scintillation counting.

Storage Buffer

♦ 20 mM Hepes (pH 7.5), 0.15 M NaCl, 1 mM EDTA, 1 mM DTT, 0.1 mg/mL BSA, and 50% (v/v) glycerol.

Storage

♦ Product is stable for at least 6 months from the date of receipt if stored at ≤-20° C in a manual defrost freezer.

♦ For long term storage, aliquot the enzyme and buffer and store at ≤ -80° C.

♦ Avoid repeated freeze-thaw cycles.

References