APE 1

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M0282S 003130315091

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1,000 units	**10,000 U/ml**	**Lot: 0031303**
RECOMBINANT	**Store at –20°C**	**Exp: 9/15**

Description: Human apurinic/apyrimidinic (AP) endonuclease, APE 1, also known as HAP 1 or Ref-1, shares homology with *Escherichia coli* exonuclease III protein. APE 1 cleaves the phosphodiester backbone immediately 5´ to an AP site, via hydrolytic mechanism, to generate a DNA single-strand break with a 3´-hydroxyl and 5´ -deoxyribose phosphate terminus. Besides AP endonuclease activity, APE 1 has also been reported to have weak DNA 3´ -diesterase, 3´ to 5´ exonuclease and RNase H activities (1–5).

In addition to DNA repair activity, APE 1 is also capable of regulating the DNA binding activity of many transcription factors *in vitro* by a redox mechanism (Ref-1). As part of this process, APE 1 has been shown to stimulate the DNA binding activity of Fos-Jun heterodimers, Jun-Jun homodimers and Hela cell AP-1 proteins as well as that of several other transcription factors including NF-kB, Myb and members of the ATF/CREB family (7–9).

Source: An *E. coli* strain which carries the cloned human APE 1 gene

Applications:

- Single cell gel electrophoresis (Comet assay)
- Alkaline elution (6)
- Alkaline unwinding (7)
- Modified nick translation

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.05 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to cleave 20 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 20 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 4 containing 20 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Recommended Dilution for the Comet Assay: 1:10^3. A detailed protocol can be found at www.neb.com

Heat Inactivation: 65°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.

SS DNA Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated [^{3}H] denatured DNA (2 x 10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.30% radioactivity.

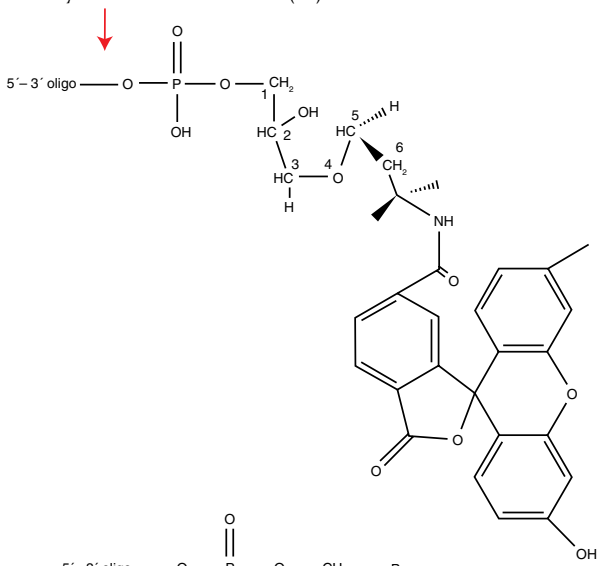
DS DNA Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated [^{3}H] DNA (2 x 10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.23% radioactivity.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl 1X Buffer 4 resulted in < 10% conversion to RF II.

References:

1. Robson, C.N. and Hickson, D.I. (1991) *Nucleic Acids Res.* 19, 5519–5523.
2. Demple, B. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 11450–11454.
3. Barzilay, G. et al. (1995) *Nucleic Acids Res.* 23, 1554–1550.
4. Barzilay, G. et al. (1995) *Nature Struc. Biol.* 2, 451–568.
5. Wilson, D.M. III et al. (1995) *J. Biol. Chem.* 270, 16002–16007.
6. Gorman, M.A. et al. (1997) *EMBO J.* 16, 6548–6558.
7. Xanthoudakis, S. et al. (1992) *EMBO J.* 11, 3323–3335.
8. Walker, L.J. et al. (1993) *Mol. Cell. Biol.* 13, 5370–5376.
9. Flaherty, D.M. (2001) *Am. J. Respir. Cell. Mol. Biol.*, 25, 664–667.
10. Vidal, A.E. (2001) *EMBO J.* 20, 6530–6539.
11. 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 APE I has been observed (11).



The structure of a 3´ fluorescently labeled oligonucleotide. Red arrow indicates where APE I cleave.

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