

APE 1



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M0282S



1,000 units **10,000 U/ml** **Lot: 0041610**
RECOMBINANT **Store at -20°C** **Exp: 4/19**

Description: Human apurinic/aprimidinic (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-κB, 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:
1:10³. 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 [³H] denatured DNA (2 x 10⁶ 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 [³H] DNA (2 x 10⁶ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.23% radioactivity.

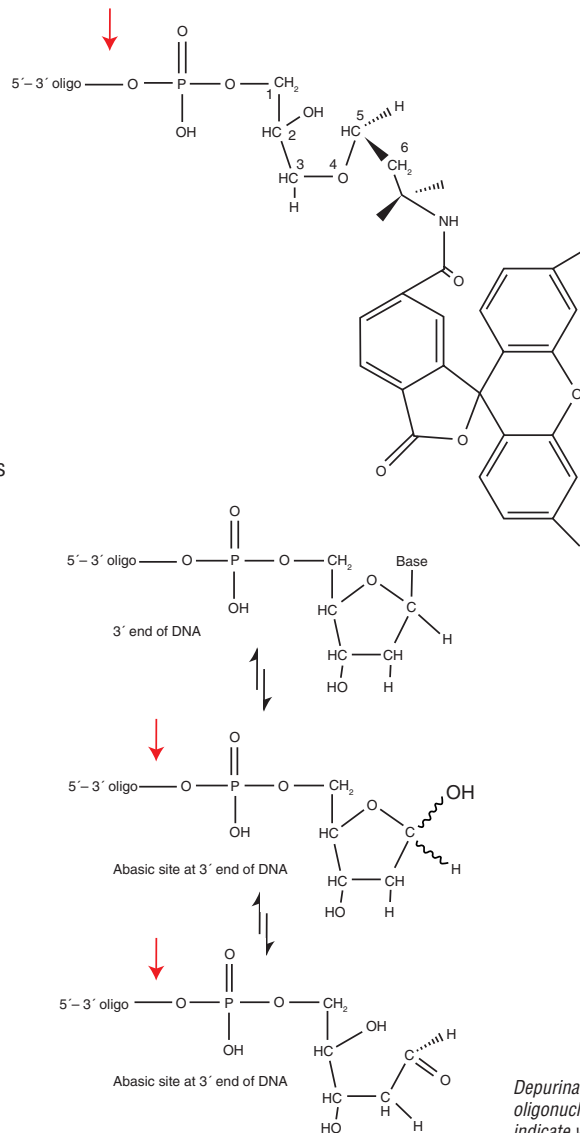
Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg φX174 RF I DNA for 4 hours at 37°C in 50 µl 1X Buffer 4 resulted in < 10% conversion to RF II.

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.
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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 1 has been observed (11).



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

Depurination/depyrimidination at the 3' end of an oligonucleotide generates an abasic site. The red arrows indicate where APE 1 may cleave.



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