



Product Information	
APE 1	
Part Number:	Y911L
Lot Number:	

Certificate of Analysis

2010-Y911-Rev.D

Product Description:

APE 1, also known as HAP 1 or Ref-1, acts as an AP lyase by hydrolyzing the phosphodiester backbone at the 5' end of an apurinic (AP) site, generating a 1 base gap in the DNA duplex and leaving 3'-hydroxyl and 5'-deoxyribose phosphate termini. Evidence suggests that APE 1 may exhibit weak DNA 3'-diesterase, 3' to 5' exonuclease and RNase H activities (1-4).

Source of Protein

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

Supplied in

10 mM Tris-HCl
50 mM NaCl
1 mM dithiothreitol
0.1 mM EDTA
50% glycerol
pH 8.0 @ 25°C

Supplied With

B012 10X Green Buffer

10X Green Buffer (B012)

200 mM Tris-Acetate
500 mM Potassium Acetate
10 mM Dithiothreitol
100 mM Magnesium Acetate
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 1 hour at 37°C.

(additional information on reverse side)

Product Specification*	
Unit Size:	10,000 Units
Unit Concentration	10,000 U/mL
Protein Concentration	0.005 mg/mL
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	2,000,000 U/mg
SS Exonuclease	10,000 U <5.0% released
DS Exonuclease	10,000 U <0.5% released
Endonuclease	10,000 U <20% converted
<i>E. coli</i> 16S rDNA Contamination	10,000 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

Quality Control Analysis:

Unit Characterization Assay

Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme batch were made in 1X reaction buffer ($[Ape1]_f = 0.2 - 0.01 \mu\text{g/mL}$) and added to 10 μL reactions containing 2.0 μM of a FAM-labeled, 34-base, duplex oligonucleotide, containing a single Uracil. [Note: substrate pre-treated for 2 minutes with 1 unit of UDG (G501L) to create an abasic site] Reactions were incubated 1 hour at 37°C, plunged on ice, denatured with N-N-dimethylformamide and analyzed on a 15% TBE-Urea acrylamide gel.

Protein Concentration (OD₂₈₀) Measurement

A 2.0 μL sample of enzyme was analyzed at OD₂₈₀ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 55,190 and molecular weight of 35,554 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)

2.0 μL of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 μL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Single-Stranded Endonuclease Activity

A 50 μ L reaction containing 11,000 cpm of a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 5.0% release of TCA-soluble counts.

Double-Stranded Endonuclease Activity

A 50 μ L reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.5% release of TCA-soluble counts.

Endonuclease Activity

A 50 μ L reaction containing 0.5 μ g of pBR322 DNA and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in 20% conversion to nicked circular DNA as determined by agarose gel electrophoresis.

***E. coli* 16S rDNA Contamination Test**

Replicate 5 μ L samples of enzyme solution were heat denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using primers for the 16S rRNA locus. The acceptance criterion for the test is the threshold cycle count (C_t) produced by the average of 3 replicate no template control samples. Based on no template control C_t values, the detection limit of this assay is <10 copies genome/sample.

Notes:

APE 1 is a DNA repair enzyme which cleaves the phosphodiester bond at abasic sites, a common form of naturally occurring DNA damage. Following thorough characterization of the APE 1 enzyme in our nuclease quality control tests, both during and after purification, we have concluded the inherent presence of abasic sites in DNA substrates contributes to false positives in tests for exogenous endo and exonuclease contaminants.

References:

1. Demple, B. et al. (1991) Proc. Natl. Acad. Sci. USA, 88, 11450-11454.
2. Barzilay, G. et al. (1995) Nucl. Acids Res., 23, 1544-1550.
3. Barzilay, G. et al. (1995) Nature Struc. Biol., 2, 451-468.
4. Unpublished observations (See "Notes" Section)



Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.