Product Information	
Endonuclease VIII	
Part Number	Y9080L
Concentration	10,000 U/mL
Unit Size	10,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: *E. coli* Endonuclease VIII functions as both an N-glycosylase (by excising oxidative base lesions) and an AP lyase (by subsequently cleaving the phosphodiester backbone), leaving terminal phosphates at the 5' and 3' ends (1). Damaged bases removed by Endonuclease VIII include: urea, 5, 6-dihydroxythymine, thymine glycol, 5-hydroxy-5-methylhydantoin, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea (1,2).

Product Specifications			
Y9080			
Assay	**SDS Purity**	**Specific Activity**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**100**
Specification	>99%	770,000 U/mg	<10 copies

Source of Protein: An *E. coli* strain carries the cloned Endonuclease VIII gene.

Unit Definition: 1 unit is defined as the amount of enzyme required to cleave 1 pmol of an oligonucleotide duplex containing a single AP site in 1 hour at 37°C.

Molecular weight: 29.8 KDa

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were prepared in Endo VIII glycerol storage solution and added to 10 µL reactions containing a FAM-labeled, 35-base, duplex oligonucleotide, containing a single Uracil (Note: substrate was pre-treated for 2 minutes with UDG to create an abasic site). Reactions were incubated 60 minutes at 37°C, plunged on ice, denatured with N-N-dimethylformamide and analyzed by running and exposing to short-wave UV a 15% TBE-Urea acrylamide gel.

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL **r**eplicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in:
10 mM Tris-HCL, 250 mM NaCl, 0.1 mM EDTA, 50% glycerol (pH 8.0 at 25°C)

Supplied with:
10X Endonuclease VIII Buffer (B9080): 100 mM Tris-HCl, 750 mM NaCl, 10 mM EDTA (pH 8.0 at 25°C)

Usage Instructions:

1. Endonuclease VIII Reaction Buffer (B9080) at 1X is recommended, but this enzyme is active in most molecular biology reaction buffers.
2. Add 0.5 µL Endonuclease VIII (Y9080) to a reaction contains DNA substrate.
3. Incubate at 37°C for 30 minutes.
4. Heat Inactivation can be carried out at 75°C for 10 minutes.

Note: protocol can be modified for application specific usage.

References:

1. Dizdaroglu, M. et al. (1993) Biochemistry, 32, 12105-12111.
2. Hatahet, Z. et al. (1994) J. Biol. Chem., 269, 18814-18820.

Disclaimer:

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