



Product Information	
<b>Endonuclease VIII</b>	
Part Number:	Y908L
Lot Number:	

Rev.C

### 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 (2,3).

### Source of Protein

An *E. coli* strain which carries the cloned Endonuclease VIII gene.

### Supplied in

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

### Supplied With:

B908 10X Endonuclease VIII Buffer

### 10X Endonuclease VIII Buffer (B908)

100 mM Tris-HCl  
750 mM NaCl  
10 mM EDTA  
pH 8.0 @ 25°C

### Unit Definition

One 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.

(additional information on reverse side)

Product Specification*	
Unit Size:	5,000
Unit Concentration	10,000 U/mL
Protein Concentration	0.013 mg/ml
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	770,000 U/mg
SS Exonuclease	100 U <0.1% released
DS Exonuclease	100 U <0.1% released
Endonuclease	100 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	100 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 Endo VIII glycerol storage solution and added to 10 µL reactions containing 2.0 µM of an FAM-labeled, 34-base, duplex oligonucleotide, containing a single Uracil. [Note: substrate pre-treated for 2 minutes with 1 unit of UDG to create an abasic site] Reactions were incubated 15 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.

#### 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 µL reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

##### Double-Stranded Endonuclease Activity

A 50 µL reaction containing 15,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

### **Endonuclease Activity**

A 50  $\mu$ L reaction containing 1  $\mu$ g of pBR322 DNA and 10  $\mu$ L of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

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

Replicate 5  $\mu$ 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. Based on no template control  $C_t$  values, the detection limit of this assay is <10 copies genome/sample.

### **References**

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



#### **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.