



Product Information	
Lambda Exonuclease	
Part Number:	X803L
Lot Number:	

Certificate of Analysis

2010-X803-Rev.D

Product Description:

Lambda Exonuclease is a highly processive exonuclease which selectively degrades the 5'-phosphorylated strand of double-stranded DNA via the stepwise 5' to 3' release of mononucleotides from duplex DNA. Lambda Exonuclease is inactive against 5'-hydroxyl termini (1), and will not initiate excision at a nick or gap (2), though it will degrade a 5'-overhanging tail from duplex DNA at a greatly reduced rate.

Source of Protein

Purified from a strain of *E. coli* that overexpresses the exonuclease gene from bacteriophage Lambda.

Supplied in

25 mM Tris-HCl
50 mM NaCl
1.0 mM Dithiothreitol
0.1% mM EDTA
50% Glycerol
pH 7.5 @ 25°C

Supplied With

B803 10X Lambda Exo Reaction Buffer

10X Lambda Exo Reaction Buffer (B803)

670 mM Glycine
25 mM MgCl₂
pH 9.4 @ 25°C

Unit Definition

One unit is defined as the amount of enzyme required to produce 10 nmol of acid-soluble deoxyribonucleotide from double-stranded substrate in 30 minutes at 37°C.

Product Specification*

Unit Size:	10,000 Units
Unit Concentration	5,000 U/mL
Protein Concentration	0.0625 mg/ml
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	80,000 U/mg
Endonuclease	1500 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	1500 U <10 copies
Storage	-20°C

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

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer ([Lambda Exo]_f = 1.25-0.01µg/mL) and added to 50 µL reactions containing a 1.1 kb tritiated DNA fragment, and 1X Lambda Exo Reaction Buffer. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using a TCA-precipitation method.

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 46,660 and molecular weight of 25,909 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)

2.0 µL of 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:

(additional information on reverse side)

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 no visually discernible 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 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. 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 the correlation between the no template control C_t values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

1. Ausubel, F. M., et al. (1987) Current Protocols in Molecular Biology (John Wiley and Sons, Inc.)
2. Little, J.W. (1981) Gene Amp. Anal., 2, 135-145

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.