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Product Information Sheet X8010L Rev D

Product Information	
Exonuclease I	
Part Number	X8010L 280430
Concentration	20,000 U/mL
Unit Size	30,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	185,000 U/mg
DS Endonuclease	200 U = no conversion
***E.coli* DNA Contamination**	200 U < 10 copies

Product Description:

Exonuclease I cleaves single-stranded DNA in the 3'→5' direction, releasing 5' -mono/di-nucleotides and leaving double-stranded DNA molecules and the 5'-terminus intact. The enzyme is processive though digestion is inhibited by the presence of a 3' -terminal phosphate. Exonuclease I is tolerant of a wide-range of buffer conditions and can typically be added to reactions containing magnesium (1-3).

Source of Protein

Purified from a strain of *E. coli* that expresses the recombinant Exonuclease I gene.

Supplied in

10 mM Tris-HCl
100 mM NaCl
1 mM DTT
0.5 mM EDTA
50% glycerol
pH 7.5 @ 25°C

Unit Definition

One unit is defined as the amount of enzyme required to produce 10 nmol of acid-soluble total nucleotide in 30 minutes at 37°C.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in a glycerol (50%) containing Exonuclease I storage solution and added to 50 µL reactions containing a single-stranded tritiated DNA fragment, and 67mM Glycine-KOH (pH 9.5), 10mM DTT, 6.7mM $MgCl_2$. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using a TCA-precipitation method.

Protein Concentration (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209), and blanked with enzyme storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 72,970 and molecular weight of 54,500 Daltons.

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.

(additional information on reverse side)

Contamination Tests:

Double-Stranded Endonuclease Activity

A 50 µL reaction containing 0.5 µg of pBR322 DNA and 10 µL 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 µL 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.

Notes:

1. Exonuclease I can be heat-inactivated by incubation at 80°C for 15 minutes.
2. Exonuclease I will preferentially degrade single-stranded oligonucleotide primers in a reaction containing amplification products or other sources of double-stranded DNA, leaving double-stranded molecules intact.
3. Exonuclease I, in combination with Lambda Exonuclease (X8030L), is effective in removing linear DNA species from plasmid preparations.
4. Exonuclease I works well in most molecular biology buffers which contain magnesium in excess of 1.5 mM.

References:

1. Lehman, I.R. and Nussbaum, A.L. (1964) *J. Biol. Chem.* **239**, 2628.
2. Kushner, S.R. *et al.* (1971) *Proc. Natl. Acad. Sci. USA* **68**, 824.
3. Kushner, S.R. *et al.* (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1366.

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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.