Product Information	
Exonuclease I	
Part Number	X8010L
Concentration	20,000 U/mL
Unit Size	30,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

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 (1,2,3).

Product Specifications				
X8010				
Assay	**SDS Purity**	**Specific Activity**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**200**	**200**
Specification	>99%	185,000 U/mg	No Conversion	<10 copies

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

Unit Definition: 1 unit is defined as the amount of enzyme required to produce 10 nmol of acid-soluble total nucleotide in 30 minutes at 37°C.

Molecular weight: 54.5 kDa

Quality Control Analysis:

Unit Activity is 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 67 mM Glycine-KOH (pH 9.5), 10 mM DTT, 6.7 mM $MgCl_2$. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using a TCA-precipitation method.

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.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL replicate 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, 100 mM NaCl, 1 mM DTT, 0.5 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

Usage Instructions: Removal of single stranded DNA from PCR products

1. Set up the following reaction mixture in a total volume of 20 µL:

- 5 µL of PCR product

- 10 units of Exonuclease I (X8010L)

2. Incubate the reaction mixture at 37°C for 15 minutes.
3. Heat inactivate enzyme at 80°C for 15 minutes.

Notes:

1. 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.
2. Exonuclease I, in combination with Lambda Exonuclease (X8030L), is effective in removing linear DNA species from plasmid preparations.
3. 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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