Product Information	
Exonuclease III	
Part Number	X8020L
Concentration	100,000 U/mL
Unit Size	50,000 U
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

Product Description: Exonuclease III is a 3'→5' exonuclease which acts by digesting one strand of a dsDNA duplex at a time or digesting the RNA strand of an RNA-DNA heteroduplex (1). Exonuclease III breaks phosphodiester bonds on the 5' side of AP sites in both dsDNA and ssDNA (2), removes 3' terminal groups on dsDNA (3), increases MutY turnover (4), and efficiently degrades 3' recessed but not 3' protruding DNA ends (creating 5' overhangs) (5). Exo III removes a limited number of nucleotides per binding event, resulting in coordinated progressive deletions within the population of DNA molecules (1).

Product Specifications					
X8020					
Assay	**SDS Purity**	**Specific Activity**	**DS Endonuclease**	***E. coli* DNA Contamination**	**UDG Activity**
Units Tested	**n/a**	**n/a**	**1000**	**1000**	**n/a**
Specification	>99%	100,000 U/mg	No Conversion	<10 copies	< 20U/mL

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

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

Molecular weight: 30,969 Daltons

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 50 µL reactions containing a tritiated DNA fragment, and 1X Exo III Yellow Buffer. 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 **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.

UDG Contamination is assessed in a 50 µL reaction containing tritiated Uracil DNA and 10 µL enzyme solution incubated for 60 minutes at 37°C under standard UDG unit characterization conditions.

Supplied in: 25mM Tris-HCl, 50mM KCl**,** 1mM DTT, 0.1mM EDTA, 50% glycerol (pH 8.0 at 25ºC).

Supplied with:
10X Yellow Buffer (B0130): 100 mM Bis-Tris-Propane-HCl, 100mM $MgCl_2$, 10mM DTT (pH 7.0 at25°C).

Usage Instructions:

1. Set up the following reaction mixture in a total volume of 50 µL:
   - 1–5 µg DNA
   - 5 µL 10x Yellow Buffer
   - 0.5 µL Exonuclease III (50 units)*
   - Nuclease-free water up to 50 µL
2. Incubate for 30 minutes at 37°C
3. Stop the reaction by adding EDTA to 10 mM final concentration and inactivate the enzyme by heating for 10 minutes at 70°C.
4. For the clean-up of the treated DNA we recommend a spin column based clean up (e.g. QIAamp DNA Micro Kit (50), Cat. No. 56304)

*Important note: In case the desired digestion result was not achieved, we recommend titrating the enzyme to identify the optimal required amount of enzyme.

References:

1. Linn, S. M. (1982) Nucleases, pp. 291-309, Cold Spring Harbor Laboratory Press.
2. Shida, T., et al. (1996) Nucl. Acids Res. 24 (22), 4572-4576.
3. Doetsch, P. W. (1990) Mutat. Res. 236 (2-3), 173-201.
4. Pope, M. A., et al. (2002) J. Biol. Chem. 277 (25), 22605-22615.
5. Henikoff, S. (1984) Gene 28, 351-359.