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Product Information Sheet X8020L Rev G

Product Information	
Exonuclease III	
Part Number	X8020L 280435
Concentration	100,000 U/mL
Unit Size	50,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	100,000 U/mg
DS Endonuclease	1000 U =No conversion
***E.coli* DNA Contamination**	1000 U <10 copies
UDG Activity	< 20U/mL activity

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 (3), 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).

Source of Protein

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

Supplied in

25 mM Tris-HCl
50 mM KCl
1.0 mM DTT
0.1 mM EDTA
50% Glycerol
pH 8.0 @ 25°C

Supplied with

B0130 (10X Yellow Buffer)

10X Yellow Buffer (B0130):

100 mM Bis-Tris-Propane-HCl
100 mM $MgCl_2$
10 mM DTT
pH 7.0 @ 25°C

Unit Definition

One unit is defined as the amount of enzyme required to produce 1 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 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}) Measurement

A 2.0 µL sample of Exonuclease III 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 Exonuclease III storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 38,690 and molecular weight of 30,969 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.

UDG Contamination Test

A 50 µL reaction containing 1 µg of tritiated Uracil containing DNA and 10 µL enzyme solution incubated for 40 minutes at 37°C under standard UDG unit characterization conditions resulted in the measurement of less than 20 U/mL UDG activity as determined by liquid scintillation analysis.

References:

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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.

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Biozym Scientific GmbH – Steinbrinksweg 27 - D-31840 Hessisch Oldendorf Tel.: +49 (0)5152-9020 - Fax: +49 (0)5152-2070 support@biozym.com www.biozym.com

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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.