

| Product Information       |                |
|---------------------------|----------------|
| <b>Lambda Exonuclease</b> |                |
| Part Number               | X8030L         |
| Concentration             | 5,000 U/mL     |
| Unit Size                 | 10,000 U       |
| Storage Temperature       | -25°C to -15°C |
| Lot Number                |                |
| Reference Number          |                |

**Product Description:** Lambda Exonuclease is a highly processive 5'→3' double-stranded exonuclease that degrades one strand of the duplex. Lambda exonuclease can initiate at blunt DNA or DNA containing 3' single-stranded overhangs. Lambda Exonuclease has greatly reduced activity on non-phosphorylated DNA, single-stranded DNA, and DNA having protruding 5' single-stranded termini. Lambda exonuclease will not initiate at a nick or gap (1,2).

| Product Specifications |            |                   |                 |                                  |
|------------------------|------------|-------------------|-----------------|----------------------------------|
| X8030                  |            |                   |                 |                                  |
| Assay                  | SDS Purity | Specific Activity | DS Endonuclease | <i>E. coli</i> DNA Contamination |
| Units Tested           | n/a        | n/a               | 1500            | 1500                             |
| Specification          | >99%       | 80,000 U/mg       | No Conversion   | <10 copies                       |

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

**Unit Definition:** 1 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.

**Molecular weight:** 25.9 kDa

**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 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 (OD<sub>280</sub>)** is determined by OD<sub>280</sub> 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:** 25 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

**Supplied with:**

**10X Lambda Exo Reaction Buffer (B8030):** 670 mM Glycine, 25 mM MgCl<sub>2</sub> (pH 9.4 at 25°C)

**Usage Instructions:** Digestion of 5' phosphorylated strand of dsDNA

1. Set up the following reaction mixture in a total volume of 50  $\mu$ L:

| Components                             | Final Concentration | Volume                      |
|----------------------------------------|---------------------|-----------------------------|
| Nuclease free water                    | N/A                 | X $\mu$ L                   |
| 10X Lambda Exo Reaction Buffer (B8030) | 1X                  | 5 $\mu$ L                   |
| DNA                                    | up to 5ug           | X $\mu$ L                   |
| Lambda Exonuclease (X8030L)            | 5 U                 | 1 $\mu$ L                   |
| <b>Total Volume =</b>                  |                     | <b>50 <math>\mu</math>L</b> |

2. Incubate at 37°C for 30 minutes.

3. Reaction can be stopped by adding 10 mM EDTA or by incubation at 75°C for 10 min.

**References:**

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.

**Disclaimer:**

Use of this enzyme in certain applications may be covered by patents and may require a license. Purchase of this product does not include a license to perform any patented application; therefore, it is the sole responsibility of the users of the product to determine whether they may be required to engage in a license agreement depending upon the particular application in which the product is used.

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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. SDS sheets relevant to this product are available upon request.