

Lambda Exonuclease

(recombinant protein)

Cat. No.	size
E1180-01	1 000 units
E1180-02	5 000 units

Unit Definition:

One unit of Lambda Exonuclease produces 10 nmoles of acid-soluble deoxribonucleotide from double-stranded DNA in 30 min at 37°C.

Storage Conditions:

Store at -20°C.

Package Contents:

- Lambda Exonuclease
- 10 x Lambda Exonuclease Buffer
- BSA [100 x], 2.5 mg/ml (added as separate component to prevent reaction buffer precipitation).

References:

1.Little, John W. (1981)Gene Amplification and Analysis 2, 135-145.

Double-stranded specific DNAse produced by *Escherichia coli* upon lambda bacteriophage infection that digests one DNA strand starting from 5' terminus.

Description:

- Double-stranded specific DNase that requires the presence of a 5'-phosphate group for activity.
- PCR products prepared using one PCR primer phosphorylated at the 5'-end and the other primer unphosphorylated can be treated with Lambda Exonuclease to yield a single-stranded DNA. That helps to obtain a clean readable sequence without the extraneous bands which are often present when PCR products are sequenced directly.
- DNA digestion with Lambda Exonuclease is especially helpful when sequencing PCR products with a high GC content.

Assay Conditions:

67 mM Glycine-KOH (pH 9.5 at 25°C), 10 mM β-mercaptoethanol, 6.7 mM MgCl₂, 25 μg/ml bovine serum albumin, 20 μg/ml sonicated [³H]-labeled Lambda DNA and Lambda Exonuclease in 50 μl for 30 min at 37°C.

10 x Reaction Buffer:

670 mM Glycine-KOH (pH 9.5 at 25°C), 100 mM β-mercaptoethanol, 67 mM MgCl₂.

Quality Control:

All preparations are assayed for contaminating nonspecific endodeoxyribonuclease and 3' exodeoxyribonuclease activities. Tested for the presence of linear DNA.

Lambda Exonuclease Digestion Protocol:

1. Mix:

Component:	Amount:
DNA	х µl (0.1 - 1 µg)
10 x Lambda Exonuclease Buffer	5 μl
100 x BSA	0.5 μl
Lambda Exonuclease	5 U
Nuclease-free Water	to 50 μl

- 2. Incubate for 15–30 min at 37°C.
- 3. Heat-inactivate (10 min, 75°C) or purify DNA by spin column purification (e.g. EURx PCR/DNA Clean-Up Purification Kit, Cat. No. E3520) or collect ssDNA by Ethanol Precipitation.

For unambiguous PCR Sequencing:

- 1. Digest purified DNA with Recombinant Lambda Exonuclease.
- 2. Heat to inactivate enzyme ready for DNA sequencing.