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Lambda Exonuclease

(Lambda bacteriophage of *Escherichia coli*)

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(Lambda bacteriophage of E. coli)

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

Unit Definition:
One unit produces 10 nmoles of acid - soluble deoxribo-nucleotide from double-stranded DNA in 30 min at 37˚C.

Storage Conditions:
Store at -20˚C

For unambiguous PCR Sequencing:

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

The sequencing primer must be in the same orientation as the 5'-phosphorylated primer, i.e. complementary to the non-digested strand.

Assay Conditions:

67 mM glycine-KOH (pH 9.4), 50 µg/ml bovine serum albumin, 2.5 mM $MgCl_2$, 20 µg/ml sonicated [^{3}H]-labeled Lambda DNA and lambda exonuclease in 50 µl for 30 min at 37˚C.

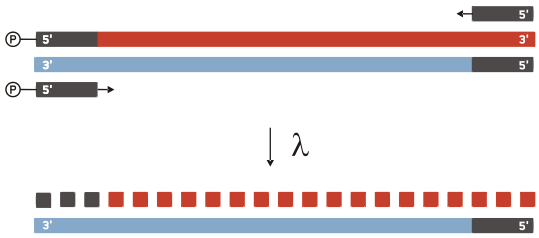
Quality Control:

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

Double-strand specific DNase produced by *Escherichia coli* upon lambda bacteriophage infection. Digests phosphorylated DNA strands starting from 5'-termini.

Description:

- Double-stranded specific DNase that strongly prefers the presence of a 5'-phosphate group to a 5'-OH group for activity. Attacks DNA exclusively from 5'-termini (1).
- Not active against nicked DNA, 5'-protruding ends and against DNA gaps. Strongly reduced activity (more than 100-fold) against long ssDNA fragments. Active against short ssDNA, blunt or 5'-recessed DNA termini (1).
- High processivity. Once bound to a DNA molecule, the enzyme continues digestion until completion, in preference to falling off and attacking another DNA molecule (1).
- PCR products amplified from one 5'-end phosphorylated PCR primer and from a second unphosphorylated primer are digested with lambda exonuclease to yield single-stranded DNA. DNA strands with unphosphorylated 5' ends are retained, while the 5'-phosphorylated strand are converted into monomers (5'-pA-OH, 5'-pC-OH, 5'-pG-OH, 5'-pT-OH).
- For preparation of ssDNA substrate for Terminal Deoxynucleotidyl Transferase (TdT), which accepts ssDNA as a more efficient substrate as compared to dsDNA (2).
- To obtain a clean readable sequence without the extraneous bands which are often present when PCR products are sequenced directly (3), or for PCR products with high GC content.
- For mutation detection with SSCP, where similar sized DNA fragments are separated on non-denaturing gels due to sequence specific secondary structure formation (4).
- For enhancing probe hybridization efficiency to DNA microarrays, thus greatly increasing signal enhancement (5).



Lambda Exonuclease Digestion Protocol:

The protocol describes digestion of 0.1 – 2 µg of DNA or 3 to 6 µl PCR product (approx. 0.1 – 1 µg DNA) where one strand is synthesized with 5'-phosphate labeled primer (substrate for digestion), and the second strand is synthesized with unlabeled primer (to be retained).

Mix

DNA	0.1 – 1 µg
10x Buffer	1.25 µl
Lambda Exonuclease	5 U
H_2O, DNase free	to 12.5 µl

Incubate for 15 – 30 min at 37˚C

Heat-inactivate (10 min, 75˚C) or purify DNA by spin column purification (e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) or collect ssDNA by Ethanol Precipitation.

References:

1. *Little, John W. (1981) Gene Amplification and Analysis 2, 135-145.*
2. *Chang, A.C.Y. et al. (1978), Nature 275, 617-624*
3. *Higuchi, R.G., Ochman, H. (1989) Nucl. Acids Res. 25, 17(14): 5865*
4. *Schwieger, F. and Tebbe C.C. (1998) Appl. Environ. Microbiol. 64, 4870-4876*
5. *Brinker et al. (2010) Biosens Bioelectron. 26 (2): 898-902*