



Exonuclease I

(*E. coli* strain that carries the cloned *Exo I* gene)

Cat. No.	size
E1150-01	4 000 units
E1150-02	20 000 units

Unit Definition:

One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C.

Storage Conditions:

Store at -20°C.

References:

1. Lehman and Nussbaum (1964) *J. Biol. Chem.*, 239, 2628.
2. Kushner, S.R. et al. (1971) *Proc. Natl. Acad. Sci. USA* 68, 824.
3. Kushner, S.R. et al. (1972) *Proc. Natl. Acad. Sci. USA* 69, 1366.
4. Goldmark and Linn (1972) *J. Biol. Chem.*, 247, 184.
5. Rosamond et al. (1979) *J. Biol. Chem.*, 254, 8646
6. Werle et al. (1994) *Nuc. Acids Research* 22(20): 4354-4355

Catalyzes the removal of nucleotides from single-stranded DNA in the 3' to 5' direction (1, 2, 3).

Description:

- Removal of residual ssDNA, including oligos, from reaction mixture (6).
- Will not degrade double-stranded DNA or RNA (4).
- Requires magnesium and presence of a free 3'-hydroxyl terminus (5).
- Active under a wide variety of buffer conditions, allowing addition of the enzyme directly into most reaction mixtures.

Assay Conditions:

67 mM Glycine-KOH (pH 9.5 at 25°C), 10 mM β-mercaptoethanol, 6.7 mM MgCl₂, 0.17 mg/ml single-stranded [3H]-DNA. Incubation is at 37°C for 10 min in a reaction volume of 50µl.

10 x Reaction Buffer:

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

10 x Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 25°C), 5 mM β-mercaptoethanol, 100 mM NaCl, 0.5 mM EDTA, 100 µg/ml BSA, 50% glycerol.

Inactivation:

Inactivated by heating at 80°C for 15 min.

Quality Control:

All preparations are assayed for contaminating RNase, endonuclease, and double-stranded exonuclease activities.

Standard PCR Clean-up Protocol:

1. Mix:

Component:	Amount:
PCR just after amplification	25-50 µl
Exonuclease I 20 U/µl	0.5 µl
Polar-BAP 5 U/µl (Cat. No. E1027)	1 µl

2. Incubate for 15 min at 37°C.
3. Heat Inactivate 15 min at 80°C.

Note 1: Up to 5 µl may be used directly to sequencing without any other purification.

Note 2: For sequencing it is recommended to use PCR without unspecific products.

Note 3: No specific buffer required.