Exonuclease I (Exo I)

Catalog Number EN0581, EN0582

Pub. No. MAN0012007 **Rev.** C.00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

Cat. No.	Contents	Amount	Storage
EN0581	Exonuclease I (Exo I)	4000 U, 20 U/µL	-25 °C to -15 °C
	10X Reaction Buffer	1 mL	
EN0582	Exonuclease I (Exo I)	20000 U, 20 U/µL	
	10X Reaction Buffer	5 x 1 mL	

Description

Exonuclease I (Exo I) degrades single-stranded DNA in a 3'→5' direction, releasing deoxyribonucleoside 5'-monophosphates in a stepwise manner and leaving 5'-terminal dinucleotides intact. It does not cleave DNA strands with terminal 3'-OH groups blocked by phosphoryl or acetyl groups (1).

Applications

- Primer removal from PCR mixtures:
  - prior to PCR product sequencing (2),
  - for one-tube "megaprimer" PCR mutagenesis (3).
- Removal of single-stranded DNA containing a 3'-hydroxyl terminus from nucleic acid mixtures.
- Assay for the presence of single-stranded DNA with a 3'-hydroxyl terminus (4).

Source

E.coli cells with a cloned *E.coli sbcB* gene.

Definition of Activity Unit

One unit of the enzyme catalyzes the release of 10 nmol of acid soluble nucleotides in 30 min at 37 °C.
Enzyme activity is assayed in the following mixture: 67 mM glycine-KOH (pH 9.5), 6.7 mM $MgCl_2$, 1 mM DTT and 0.17 mg/mL single-stranded [^{3}H]-DNA.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50 % (v/v) glycerol.

10X Reaction Buffer

670 mM glycine-KOH (pH 9.5 at 25 °C), 67 mM $MgCl_2$, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: 20 % (w/v) PEG 8000 (5).
- Inactivated by heating at 80 °C for 15 min.

Note

The enzyme is not suitable for removing 3'-overhangs of dsDNA.

Protocol for PCR product clean-up prior to sequencing

The clean-up reaction removes unincorporated primers and degrades unincorporated nucleotides. The resulting PCR product is ready to use for sequencing without additional purification, e.g., using column purification kits.

1. Prepare the following reaction mixture:

Components	Volume
PCR mixture (directly after completion of PCR)	5 µL
Exonuclease I	0.5 µL (10 U)
Thermo Scientific™ FastAP™ Thermosensitive Alkaline Phosphatase (#EF0651)	1 µL (1 U)

2. Mix well and incubate at 37 °C for 15 min.
3. Stop the reaction by heating the mixture at 85 °C for 15 min.

Note

- Up to 5 µL of purified PCR products can be used directly for DNA sequencing without further purification.
- For reliable sequencing results there should not be nonspecific PCR products.
- The protocol may be applied for clean-up of PCR products, generated by any thermophilic DNA polymerase or polymerase mix.
- The procedure is not recommended for downstream cloning applications.

Reference

1. Lehman, I.R., Nussbaum A.L., The deoxyribonucleases of *Escherichia coli*. V. On the specificity of exonuclease I (phosphodiesterase), J. Biol. Chem., 239, 2628-2636, 1964.
2. Werle, E., et al., Convenient single-step, one tube purification of PCR products for direct sequencing, Nucleic Acids Res., 22, 4354-4355, 1994.
3. Nabavi S., Nazar R.N., Simplified one tube “megaprimer” polymerase chain reaction mutagenesis, Anal Biochem., 2, 346-348, 2005.
4. Rosamond, J., et al., Modulation of the action of the recBC enzyme of *Escherichia coli* K-12 by Ca^{2+}, J. Biol. Chem., 254, 8646-8652, 1979.
5. Sasaki, Y., Miyoshi, D. and Sugimoto, N., Regulation of DN nucleases by molecular crowding., Nucleic Acids Res., 35, 4086-4093, 2007.

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