Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Exonuclease I (Exo I)

#EN0582 20000 u

Lot: **Expiry Date:**

Concentration: 20 u/µl

Supplied with: 5 x 1 ml of 10X Reaction Buffer

Store at -20°C

In total 6 vials.

ISO 9001 ISO 14001
www.fermentas.com

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:

PCR mixture (directly after completion of PCR)	5 µl
Exonuclease I	0.5 µl (10 u)
FastAP™ Thermosensitive Alkaline Phosphatase (#EF0651) *or* **Shrimp Alkaline Phosphatase** (#EF0511)	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.

(continued on back page)

QUALITY CONTROL ASSAY DATA

Double-stranded Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 100 units of Exonuclease I with 1 µg of pUC19 DNA in 50 µl of reaction buffer for 16 hours at 37°C.

Single-stranded Endodeoxyribonuclease Assay

No decrease in the amount of closed circular DNA was observed after incubation of 100 units of Exonuclease I with 1 µg of M13mp19 single-stranded DNA in 50 µl of reaction buffer for 16 hours at 37°C.

Double-stranded Exodeoxyribonuclease Assay

No detectable degradation of pUC19 DNA/AluI fragments was observed after incubation of 25 units of Exonuclease I with 1 µg of digested DNA in 50 µl of reaction buffer for 4 hours at 37°C.

Ribonuclease Assay

≤0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of Exonuclease I with 1 µg of [^{3}H]-RNA in 50 µl of reaction buffer for 16 hours at 37°C.

Quality authorized by:  Jurgita Zilinskiene

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.

The purchase of this product allows the purchaser to use it for preparing amplified DNA fragments under a license from GE Healthcare of U.S. Patent Nos. 5,741,676 and 5,756,285 and other foreign patents.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.