Exonuclease I

GE014.0001 – 20.000U (20U/µl)

Exonuclease I (Exo I) is an exonuclease that hydrolyzes single-stranded DNA, one nucleotide at a time from the end, in the 3´→5´ direction. It releases 5´- mononucleotides one after another and leaves the terminal 5´-dinucleotide intact. This 55kDa enzyme, product of the *E.coli sbcB* gene, does not cleave DNA strands without terminal 3´-hydroxyl groups, as these are blocked by phosphoryl or acetyl groups[1]. It does not degrade double-stranded DNA. For activity, Exo I requires magnesium (optimal [Mg^{2+}] is 10mM). Exo I is tolerant to a wide variety of buffer conditions (salt, pH, etc), and thus can be added directly to most molecular biology buffers containing magnesium (>1mM), including PCR reaction mixtures.

Quantity and Specifications

Specific Activity: 20U/µl. Purified from a recombinant *E.coli* strain harbouring the *sbcB* gene. Product comprises of 20.000U of Exonuclease I in 1ml of 10mM Tris-HCl pH7,5 (25ºC), 100mM NaCl, 1mM DTT, 0.5mM EDTA and 50% glycerol. Exo I is supplied with 1,5ml of 10x reaction buffer consisting of 670mM Glycine-KOH pH9,5 (25ºC), 67mM $MgCl_2$ and 100mM ß-mercaptoethanol. RNAse-, endonuclease-, and double-stranded exonuclease activities are not detectable.

Units

One Unit (1 U) is defined as the amount of enzyme required to catalyze the release of 10nmol of acid-soluble nucleotides from 0.17mg/ml ssDNA in 50µl 1x reaction buffer during incubation at 37ºC in 30 minutes.

Storage and Shelf Life

Exo I and 10x reaction buffer can be stored for up to 2 years at -20ºC.

Quality Control

Functionally tested for digestion of ssDNA. Absence of ribonuclease, and endonuclease activity was confirmed by appropriate assays.

1) Lehman, IR., and Nussbaum, AL. (1964) *J.Biol.Chem*. **239**: 2628-2636

Inhibition

Exonuclease I can tolerate a wide variety of buffers and only a few common molecular biology compounds are known to inhibit Exo I. PEG8000 at high concentrations (20% (w/v)) inhibits Exonuclease I due to molecular crowding.

Inactivation

Exonuclease I can be heat-inactivated by incubation at 80ºC for 15 minutes.

Applications

Removal of primers from PCR reaction mixtures prior to DNA sequencing or DNA labelling (typically in a PCR Clean-up protocol in combination with the use of Shrimp Alkaline Phosphatase[2] (SAP)). Removal of linear DNA molecules from plasmid preparations (in combination with the use of Lambda Exonuclease). Removal of ssDNA containing a 3´-hydroxyl group from heterogeneous mixtures of nucleic acids. Assay for the presence of ssDNA regions.

General Protocol

1) Set-up the reaction (50µl) as follows:
    - DNA up to 1 µg
    - Reaction Buffer (10X) 5 µl
    - Exo I (20U/µl) 1 µl
    - Ultrapure water up to 50 µl
2) Incubate at 37ºC for 1 - 4 hours.
3) Heat-inactivate at 80ºC for 15 min.

Protocol for PCR Clean-up (prior to DNA sequencing)

1) Add 0,5-2,0U of rSAP (#GE015.0001) and 10-20U of Exo I directly to 5 µl of PCR reaction mixture and incubate at 37ºC for 15min.
2) Heat-inactivate at 80ºC for 15 min and use 5µl of purified PCR product directly for DNA sequencing. There is no need for further purification.

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2) Werle, E. *et al.* (1994) *Nucleic Acids Res*. **22**: 4354-4355