Exonuclease I

#GE014.0001 (1ml)
(FOR RESEARCH ONLY)

Product: 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 recombinant enzyme, produced in *E.coli*, does not cleave DNA strands without terminal 3´-hydroxyl groups, as these are blocked by phosphoryl or acetyl groups. It does not degrade double-stranded DNA. For activity, Exo I requires magnesium. 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. Optimal reaction is at 0-15mM $MgCl_2$ and ≤ 100mM NaCl at 25-37ºC.

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 (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.

Content: #GE014.0001 contains 1 ml of Exonuclease I at a concentration of 20U/µl (20,000U in total), supplied in 10mM Tris-HCl pH 7.5 (25ºC), 200mM NaCl, 10mM $MgCl_2$, 1mM DTT, 1µM EDTA, 0.01% Triton X-100 and 50% glycerol

Properties: Free of RNases and endonucleases.
Specific Activity: >20U/µl. (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 reaction buffer during incubation at 25ºC in 30 minutes.)

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

Storage: Exonuclease I can be stored for up to 3 years at -20ºC. Enzyme stability is not affected by up to 5 freeze-thaw cycles.

Usage:

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 1 min (or at 60ºC for 15 min).

* for example: 200mM Tris-HCl pH 7.5, 1M NaCl, 100mM $MgCl_2$, 10 mM DTT, 0.1% Triton X-100

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

1) Add 0.5-2.0U of rSAP (**#GE015**) 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 5 minutes and use 5µl of purified PCR product directly for DNA sequencing. There is no need for further purification.

Inactivation

GRiSP's Exonuclease I is heat-sensitive and can be inactivated by incubation at 80ºC for 1 minute or at 60ºC for 15 minutes.