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Exonuclease I, *E. coli*

Cat. No. X40520K

1. Introduction

Exonuclease I (Exo I), the product of the sbcB gene of *E. coli*, is an exodeoxyribonuclease that hydrolyzes single-stranded DNA (ssDNA) stepwise in a 3′→5′ direction.[1-3] Hydrolysis generates deoxyribonucleoside 5′ monophosphates and a terminal dinucleotide diphosphate.[1] The enzyme requires magnesium (optimal Mg^{2+} concentration is 10 mM) and the presence of a free 3′-hydroxyl terminus.[1] Exo I is active under a wide variety of buffer conditions, allowing addition of the enzyme directly into most reaction mixes. Heat inactivation results from incubation at 80°C for 15 minutes.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
Exonuclease I, *E. coli*	20,000 Units	X40520K	Exonuclease I (20 Units/µL)	E0027-20D1	1 mL

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: Exo I is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM dithiothreitol, 0.1 mM EDTA, and 0.1% Triton® X-100.

Unit Definition: One unit of Exo I results in the acid-solubilization of 10 nmol of nucleotides from calf thymus DNA in 30 minutes at 37°C.

Quality Control: Exo I is function-tested in a reaction containing 33 mM Tris-acetate (pH 7.5), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM dithiothreitol, 10 µg of denatured calf thymus DNA, and varying amounts of Exo I.

Contaminating Activity Assays: Exo I is free of detectable RNase, endonuclease, and double-stranded exonuclease activities.

4. Applications

Removal of residual ssDNA and oligonucleotides from reaction mixes. Linear ssDNA and oligonucleotides can be selectively degraded from heterogeneous mixtures of nucleic acids in reaction mixes.

Clean up of PCR. Unused amplification primers can be removed after PCR using Exo I.

Removal of ssDNA from nucleic acid mixtures. Linear ssDNA can be selectively degraded from heterogeneous mixtures of nucleic acids with Exo I.

Assay for regions of ssDNA.[4,5] Use Exo I to assay for the presence of ssDNA containing a free 3′-hydroxyl end. This technique was used to characterize the endonuclease and helicase activities of purified *recBC* protein on circular fd phage DNA and duplex phage T7 DNA respectively.

5. References

1. Lehman, I.R. and Nussbaum, A.L. (1964) *J. Biol. Chem.* **239**, 2628.
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4. Goldmark, P.J. and Linn, S. (1972) *J. Biol. Chem.* **247**, 1849.
5. Rosamond, J. *et al.*, (1979) *J. Biol. Chem.* **254**, 8646.