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Exonuclease III, *E. coli*

Cat. Nos. EX4405K and EX4425K

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1. Introduction

Exonuclease III (Exo III) is an exodeoxyribonuclease that digests one strand of duplex DNA from a blunt end, 5′ overhang or nick.[1,2] The enzyme acts in a 3′→5′ direction producing stretches of single-stranded (ss) DNA.[1,2] Exo III is not active on 3′ overhangs of 4 or more bases in length, ssDNA, or on thioester-linked nucleotides.[2] The enzyme also has RNase H, 3′-DNA phosphatase, and apurinic DNA endonuclease, activities.[2,3] Exo III can be heat inactivated by incubation at 65°C for 15 minutes.

Exo III digestions proceed at a uniform rate yielding predictable and reproducible results. However, the observed rate of nucleotide excision can vary depending on the individual characteristics of the reaction mix. These include reaction temperature, ionic strength, template base content, template helical structure, and enzyme-to-DNA ratios.[3,4,5]

Exo III is available in 5,000- and 25,000-Unit sizes at a concentration of 200 Units/µl. 10X Reaction Buffer is provided with the enzyme.

2. Applications

Generation of nested sets of unidirectional deletions in combination with Mung Bean Nuclease.[3,6] Target DNA is double-restricted such that a 5′-overhang or blunt end is positioned adjacent to the sequences to be deleted, and a 3′ overhang is positioned adjacent to the sequences to be protected from digestion. Exo III will only digest from the 3′ end of the 5′-overhang/blunt terminus. Subsequently, single-stranded regions are then removed with Mung Bean Nuclease and plasmids are recircularized with T4 DNA Ligase.

Preparation of single-stranded templates for di-deoxynucleotide sequencing.[3,6] Templates may be generated directly from linearized duplex DNA where one strand is selectively degraded with Exo III. Templates may also be generated from sets of nested deletion subclones in vectors such as M13 derivatives or phagemids produced as described above.

Production of intermediates for site-directed mutagenesis.[3,7,8] Exo III digestion of randomly introduced apurinic sites can be used as insertion sites for linker-scanning mutant production. A mutant primer is hybridized to a ssDNA template and extended in the presence of 5′-methyl-dCTP. Subsequent digestion with a methylation-sensitive restriction enzyme results in nicking of the unmethylated template strand. Exo III then selectively removes the template strand leaving mutant ssDNA.

Production of strand-specific probes.[3] Duplex DNA can be made entirely or partially single-stranded by Exo III digestion. The single-strand then serves as a template for primer-dependent, strand-specific probe synthesis during an extension reaction.

3. Product Specifications

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

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

Unit Definition: One unit of Exo III results in the acid-solubilization of 1 nmol of nucleotides from calf thymus DNA in 30 minutes at 37°C.

Quality Control: Exo III 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 DTT, 500 µg of denatured calf thymus DNA, and varying amounts of enzyme.

10X Reaction Buffer: 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5.0 mM DTT.

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

4. Related Products

The following products are also available:

– Exonuclease I, *E. coli*
– Mung Bean Nuclease
– EZ-Tn5™ Plasmid-Based Deletion Machine
– pWEB-TNC™ Deletion Cosmid Transposition Kit

5. References

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