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roboklon

Exonuclease III

(*Escherichia coli*)

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Cat. No.	Size
E1140-01	25 000 units
E1140-02	125 000 units

Unit Definition:
One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble radioactivity in 30 min at 37°C (6).

Storage Conditions:
Store at -20°C

Exonuclease III is a 3'→5' exonuclease, releasing 5'-mononucleotides from the 3'-ends of DNA strands.

Description:

- The 3'→5' exonuclease is specific towards double-stranded DNA.
- Contains DNA 3'-phosphatase, hydrolyzing 3'-terminal phosphomonoesters.
- Contains AP endonuclease, cleaving phosphodiester bonds at apurinic or apyrimidinic sites to produce 5'-termini that are base-free deoxyribose 5'-phosphate residues (1).
- The enzyme has ribonuclease H activity, preferentially degrading the RNA strand in a DNA-RNA hybrid duplex, presumably exonucleolytically (1).
- Exonuclease III digests duplex DNA at nicks producing single-stranded gaps.
- Will not degrade double-stranded DNA with 3' overhang of at least 4 base pairs, single-stranded DNA or phosphorothioate-linked nucleotides.
- Ultrapure recombinant enzyme.
- Applications of the enzyme:
  - construction of nested unidirectional deletions of DNA fragments (2)
  - generation of a single-stranded template for dideoxy-sequencing of DNA (3)
  - site-directed mutagenesis (4) and cloning of PCR products (5).

Storage Buffer:

25 mM Tris-HCl (pH 8.0 at 22°C), 0.05 mM dithiothreitol and 50% [v/v] glycerol.

Assay Conditions:

50 mM Tris-HCl (pH 7.6 at 22°C), 10 mM $MgCl_2$, 1 mM dithiothreitol and 1.5 nM duplex [^{3}H] lambda DNA. Incubation is at 37°C for 30 min in a reaction volume of 20 µl.

Quality Control:

All preparations are assayed for contaminating endonuclease activity. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

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2. *Henikoff, S., Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing, Gene, 28, 351-359, 1984.*
3. *Guo, Li-He., Wu, R., New rapid methods for DNA sequencing based on exonuclease III digestion followed by repair synthesis, Nucleic Acids Res., 10, 2065-2084, 1982.*
4. *Vandeyar, M.A., et al., A simple and rapid method for the selection of oligodeoxynucleotide-directed mutants, Gene, 65, 129-133, 1988.*
5. *Li, Ch., Evans, R.M., Ligation independent cloning irrespective of restriction site compatibility, Nucleic Acids Res., 25, 4165-4166, 1997.*
6. *Richardson, C. C.,Lehman, I. R. and Kornberg, A. (1964) J. Biol. Chem.239,251-258.*