



## Exonuclease III

(*Escherichia coli*)

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 radio-activity in 30 min at 37°C (6).

### Storage Conditions:

Store at -20°C.

### References:

1. Rogers, S.G. and Weiss, B., Exonuclease III of *Escherichia coli* K-12, an AP endonuclease, *Methods Enzymol.*, 65, 201-211, 1980.
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.

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

### Description:

- The 3'->5' exonuclease 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).

### Assay Conditions:

50 mM Tris-HCl (pH 7.6 at 22°C), 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol and 1.5 nM duplex [<sup>3</sup>H] Lambda DNA.

Incubation is carried out at 37°C for 30 min in a reaction volume of 20 µl.

### Storage Buffer:

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

### Quality Control:

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

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

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