Exonuclease III

*(Escherichia coli)*

**Exonuclease III**

**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 MgCl₂, 1 mM dithiothreitol and 1.5 mM duplex [³H] 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:**

**Cat. No.**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Size</th>
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<tbody>
<tr>
<td>E1140-01</td>
<td>25 000 units</td>
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<tr>
<td>E1140-02</td>
<td>125 000 units</td>
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**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.