# Exonuclease III
## *(E. coli)*

NEW ENGLAND BioLabs Inc.
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M0206S 033150417041

# M0206S

RRR NEB1 Yes!

**5,000 units** **100,000 U/ml** **Lot: 0331504**
**RECOMBINANT** **Store at –20°C** **Exp: 4/17**

**Description:** Catalyzes the stepwise removal of mononucleotides from 3´-hydroxyl termini of duplex DNA (1). A limited number of nucleotides are removed during each binding event, resulting in coordinated progressive deletions within the population of DNA molecules (2).

The preferred substrates are blunt or recessed 3´-termini, although the enzyme also acts at nicks in duplex DNA to produce single-strand gaps. The enzyme is not active on single-stranded DNA, and thus 3´-protruding termini are resistant to cleavage. The degree of resistance depends on the length of the extension, with extensions 4 bases or longer being essentially resistant to cleavage. This property can be exploited to produce unidirectional deletions from a linear molecule with one resistant (3´-overhang) and one susceptible (blunt or 5´-overhang) terminus (3).

Exonuclease III activity depends partially on helical structure (4) and displays sequence dependence (C>A=T>G; ref. 5). Temperature, salt concentration and the ratio of enzyme to DNA greatly affect enzyme activity, requiring reaction conditions to be tailored to specific applications.

Exonuclease III has also been reported to have RNase H, 3´-phosphatase and AP-endonuclease activities (1).

**Source:** Purified from *E. coli* K-12, BE257/pSGR3 strain (kindly supplied by B. Weiss)

**Applications:**
- Unidirectional nested deletions (3)
- Site-directed mutagenesis (6)
- Preparation of strand-specific probes (2)
- Preparation of single-stranded substrates for dideoxy sequencing (7)

Supplied in: 200 mM KCl, 5 mM $KPO_4$ (pH 6.5), 0.05 mM EDTA, 5 mM 2-mercaptoethanol, 200 µg/ml bovine serum albumin and 50% glycerol. Store at –20°C.

**Reagents Supplied with Enzyme:**
10X NEBuffer 1.

**Reaction Conditions:**
1X NEBuffer 1.
Incubate at 37°C.

**1X NEBuffer 1:**
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.0 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to produce 1 nmol of acid soluble total nucleotide in 30 minutes at 37°C in a total reaction volume of 50 µl.

**Unit Assay Conditions**: 1X NEBuffer 1, 0.15 mM sonicated duplex $^3H$ DNA and enzyme for 30 minutes at 37°C in a total reaction volume of 50 µl.

## Quality Control Assays

**Endonuclease Activity:** Incubation of 250 units of Exonuclease III with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 50% conversion to RF II.

**Heat Inactivation:** 70°C for 20 minutes.

Phosphorothioate linkages are not cleaved by Exonuclease III. Unidirectional deletions can also be created by protecting one terminus by incorporation of α-phosphorothioate-containing nucleotide (8).

*(See other side)*

CERTIFICATE OF ANALYSIS

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**References:**


1. Rogers, G.S. and Weiss, B. (1980). In L. Grossman and K. Moldave (Eds.), *Methods in Enzymology Vol.* 65, (pp. 201–211). New York: Aceademic Press.
2. Sambrook, J. et al. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed, (pp 5.84–5.85). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
3. Henikoff, S. (1984) *Gene* 28, 351–359.
4. Richardson, C. C. et al. (1964) *J. Biol. Chem.* 239, 251–258.
5. Linxweiler, W. and Horz, W. (1982) *Nucl. Acids Res.* 10, 4845–4859.
6. Vandeyar, M. A. (1988) *Gene* 65, 129–133.
7. Guo, L. H. and Wu, R. (1982) *Nucl. Acids. Res.* 10, 2065–2084.
8. Putney, S., Benkovic, S. and Schimmel, P. (1981) *Proc. Natl. Acad. Sci. USA* 78, 7350–7354.




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Page 2 (M0206)