

## Exonuclease VIII, truncated



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M0379S 001150617061

# M0545S



**1,000 units**    **10,000 units/ml**    **Lot: 0011506**  
**RECOMBINANT**    **Store at -20°C**    **Exp: 6/17**

**Description:** Exonuclease VIII, truncated, is a genetic engineering active domain of exonuclease VIII from *E. coli*. Exonuclease VIII, truncated is able to initiate nucleotide removal from the 5' termini of linear double-stranded DNA in the 5' to 3' direction (1). However, the enzyme does not degrade supercoiled dsDNA and circular ssDNA.

**Source:** An *E. coli* strain that carries a plasmid with genetic engineering active domain of Exonuclease VIII.

### Application:

- Degradation of linear dsDNA while maintaining double stranded circular DNA.

Supplied in: 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT and 0.1% Triton™ X-100 and 50% glycerol.

**Reagents Supplied with Enzyme:**  
NEBuffer 4 (10X)

**Reaction Conditions:** 1X NEBuffer 4.  
Incubate at 37°C.

**1X NEBuffer 4:**  
20 mM Tris-acetate  
50 mM Potassium Acetate  
10 mM Magnesium Acetate  
1 mM DTT  
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [<sup>3</sup>H] DNA.

**Unit Assay Conditions:** 1X NEBuffer 4 with 0.15 mM with sonicated duplex <sup>3</sup>H- DNA.

### Quality Control Assays

**Endonuclease Activity (Nicking):** A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled PhiX174 RF I DNA and a minimum of 50 units of Exonuclease VIII incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Endonuclease Activity (Circular Single Stranded DNA):** A 50 µl reaction in NEBuffer 4 containing 1 µg of M13mp18 Single-stranded DNA and a minimum of 30 units of Exonuclease VIII incubated for 4 hours at 37°C results in < 10% conversion to linear DNA as determined by agarose gel electrophoresis.

**Endonuclease Activity (Nicked Circular DNA):** A 50 µl reaction in NEBuffer 4 containing 1 µg of PhiX174 RF II DNA and a minimum of 30 units of Exonuclease VIII incubated for 4 hours at 37°C results in < 10% conversion to linear DNA as determined by agarose gel electrophoresis.

**Protein Purity Assay (SDS-PAGE):** Exonuclease VIII is > 95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

**RNase Activity (Extended Digestion):** A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 10 units Exonuclease VIII is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

**Heat Inactivation:** 15 minutes at 70°C.

### References:

- Chang et al. (2001) *JBC* 46004–46010.



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