Exonuclease VIII, truncated

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M0545S 003170319031

M0545S

1,000 units **10,000 units/ml** **Lot: 0031703**
RECOMBINANT **Store at –20°C** **Exp: 3/19**

Description: Exonuclease VIII, truncated, is a genetically engineered 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). 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 [^{3}H] DNA.

Unit Assay Conditions: 1X NEBuffer 4 with 0.15 mM with sonicated duplex ^{3}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:

1. Chang, et al. (2001) *J. Biol. Chem.* 46004–46010.

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CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS