T7 Exonuclease

NEW ENGLAND BioLabs Inc.
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M0263S 002120514051

M0263S

1,000 units **10,000 U/ml** **Lot: 0021205**
RECOMBINANT **Store at –20°C** **Exp: 5/14**

Description: T7 Exonuclease acts in the 5´ to 3´ direction, catalyzing the removal of 5´ mononucleotides from duplex DNA. T7 Exonuclease is able to initiate nucleotide removal from the 5´ termini or at gaps and nicks of double-stranded DNA (1). It will degrade both 5´ phosphorylated or 5´ dephosphorylated DNA. It has been also reported to degrade RNA and DNA from RNA/DNA hybrids in the 5´ to 3´ direction but is unable to degrade either double-stranded or single-stranded RNA (2). The protein is the product of T7 gene 6.

Source: Purified from an *E. coli* strain containing a TYB12 intein fusion

Supplied in: 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 5 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions:
1X NEBuffer 4.
Incubate at 25°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme to produce 1 nmol of acid soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 25 °C

Unit Assay Conditions: 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1mM dithiothreitol (pH 7.9) and 0.15 mM sonicated duplex [^{3}H] DNA.

Quality Control Assays

SS DNA Exonuclease Activity: Incubation of 10 units of enzyme with 1 µg sonicated and denatured [^{3}H]-DNA (10^5 cpm/µg) for 30 minutes at 25°C in 50 µl reaction buffer released < 1.5% radioactivity.

Endonuclease Activity: Incubation of 400 units of enzyme with 1 µg ϕX174 RF I DNA for 1 hour at 25°C in 50 µl 1X reaction buffer resulted in < 10% conversion to RF II.

RNase Activity (RNA/DNA Hybrid): Incubation of 10 units of enzyme with 15.2 nmol [^{3}H]poly(A)•poly(dT) hybrid polymer for 1 hour at 37°C in a 50 µl reaction buffer released 9.7 nmol adenosine - 5´-monophosphate.

Heat Inactivation: No

References:

1. Kerr, C. and Sadowski, P. D. (1972) *J. Biol. Chem.* 247, 305–318.
2. Shinozaki, K. and Okazaki, T. (1978) *Nucleic Acids Res.* 5, 4245–4261.

CERTIFICATE OF ANALYSIS

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