T7 Exonuclease

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.

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, 1 mM dithiothreitol (pH 7.9) and 0.15 mM sonicated duplex [3H] DNA.

Quality Control Assays
Single Stranded Deoxyribonuclease Activity
(FAM Labeled Oligo): A 50 µl reaction in NEBuffer 4 containing a 50 nM solution of a fluorescent internal labeled oligonucleotide at a minimum of 50 units of T7 Exonuclease incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction containing 100 units of T7 Exonuclease with 1 µg of φX174 RF I DNA for 4 hours at 25°C resulted in < 10% loss in supercoiled DNA as determined by agrose gel electrophoresis.

Functional Assay (RNase, RNA/DNA Hybrid): Incubation of 10 units of T7 Exonuclease with 20 nmol [3H]poly(A).poly(dT) hybrid polymer for 1 hour at 37°C in a 50 µl reaction released 15 nmol adenosine-5-monophosphate.

Physical Purity: Purified to > 95% homogeneity 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 fluorescein labeled RNA transcript and 10 units of T7 Exonuclease incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescence detection.

Heat Inactivation: No

References: