T7 Exonuclease

**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: 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT. pH 7.9 @ 25°C

Incubate at 25°C.

1X NEBuffer 4: 100 mM potassium acetate, 50 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9) and 0.15 mM sonicated duplex [3H] DNA.

**Quality Control Assays**

**Endonuclease Activity:** Incubation of a 50 µl reaction containing 100 units of T7 Exonuclease with 1 µg of φX174 RF I DNA for 2 hours at 25°C resulted in <10% loss in supercoiled DNA as determined by capillary 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.

**Quality Control Assays**

**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 capillary 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:**