**Exonuclease VII**

**M0379S**  200 units  10,000 units/ml  Lot: 0010909  RECOMBINANT  Store at −20°C  Exp: 8/11

**Description:** Exonuclease VII, (Exo VII) derived from *E. coli*, cleaves single-stranded DNA (ssDNA) from both 5´→3´ and 3´→5´ direction. This enzyme is not active on linear or circular dsDNA (1,2). It is useful for removal of single stranded oligonucleotide primers (3) from a completed PCR reaction when different primers are required for subsequent PCR reactions. Digestion of ssDNA by Exonuclease VII is metal-independent.

**Source:** An *E. coli* strain that carries cloned Exonuclease VII (XseA and XseB) genes from *E. coli*.

### Applications:
- Removal of single-stranded oligonucleotide primers after PCR (3)
- Removal of terminal phosphorothioated ss-oligonucleotide primers after PCR
- Mapping positions of introns in genomic DNA (4)
- Removal of single-stranded DNA from dsDNA

**Supplied in:** 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 0.1 M NaCl and 0.1% Triton™ X-100. (pH 7.5 @ 25°C).

**Reagents Supplied with Enzyme:** 5X Exonuclease VII Reaction Buffer.

**Reaction Conditions:** 1X Exonuclease VII Reaction Buffer. Incubate at 37°C.

1X **Exonuclease VII Reaction Buffer:**
- 50 mM Tris-HCl
- 50 mM sodium phosphate
- 10 mM 2-mercaptoethanol
- 8 mM EDTA
(pH 8.0 @ 25°C)

**Unit Definition:** One unit is defined as the amount of enzyme that will catalyze the release of 1 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C.

**Unit Assay Conditions:** 1X Exonuclease VII Reaction Buffer containing 0.15 mM sonicated single-stranded [³H] *E. coli* DNA.

**Quality Control Assays**

16-Hour Incubation: A 50 µl reaction in NEBuffer 4 containing 1 µg of HaeIII-cut φX174 Phage DNA and 10 units of Exonuclease VII incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

ds-Exonuclease Activity: Incubation of a 50 µl reaction containing 10 units of Exonuclease VII in NEBuffer 4 with 1 µg of double-stranded [³H] *E. coli* DNA (10⁵ cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

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

**Physical Purity:** Purified to > 99% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(see other side)
qPCR DNA Contamination (*E. coli* Genomic):
A minimum of 10 units of Exonuclease VII is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is less than 1 copy of *E. coli* genome.

**Heat Inactivation:** 95°C for 10 minutes.

**References:**