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

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.

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 Haelll-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 (10^5 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 units 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.
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: