Exonuclease VII

**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-Cl (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-Cl
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 [³²P] E. coli DNA.

**Quality Control Assays**

**ds-Endonuclease Activity:** Incubation of 50 units of Exonuclease VII with 1 µg of λX174 RF I DNA for 4 hours at 37°C in NEBuffer 4 resulted in <10% conversion to RF II as determined by agarose gel electrophoresis.

**ss-Endonuclease Activity:** Incubation of 10 units of Exonuclease VII with 1 µg of M13 ssDNA for 1 hour at 37°C in NEBuffer 4 resulted in <20% decrease in the amount of closed circular DNA as determined by agarose gel electrophoresis.

**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.

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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: