Exonuclease VII

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.

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 (106 cpm/μg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

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 Haelli-cutt 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.

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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.

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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: