Exonuclease VII

1-800-632-7799
info@neb.com
www.neb.com

M0379S

200 units **10,000 units/ml** **Lot: 0011506**
RECOMBINANT **Store at –20°C** **Exp: 6/17**

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 [^{3}H] *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 [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

ds-Endonuclease Activity: Incubation of 50 units of Exonuclease VII with 1 µg ϕX174 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 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)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

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:

1. Chase, et al. (1974) *J. Biol. Chem.* 249, 4545–4552.
2. Chase, et al. (1974) *J. Biol. Chem.* 249, 4553–4561.
3. Li, H. et al. (1991) *Nucl. Acids Res.* 19, 3139–3141.
4. Berk, A.J. et al. (1978) *Proc. Natl. Acad. Sci. USA*, 75, 1274–1278.

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Page 2 (M0379)