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BioVision

Exonuclease I

CATALOG #: 9217 (500 UN)

ALTERNATE NAMES: Exo I

SOURCE: *E. coli* strain containing an overproducing clone of *E. coli* Exonuclease I.

PURITY: Greater than 95% pure as determined by SDS PAGE. Tested for contaminating endonucleases, double-stranded exonucleases, and ribonucleases.

MOL. WEIGHT: 55 kDa

CONCENTRATION: Standard Conc.: 10 units/µl

OPTIMUM TEMPERATURE: 37 °C

UNIT DEFINITION: One unit is the amount of enzyme which catalyzes the release of 10 nmol of acid-soluble nucleotide from denatured DNA in 30 min at 37°C under standard conditions.

STORAGE BUFFER: 20 mM Tris-HCl (pH 7.5), 5 mM 2-mercaptoethanol, 0.5 mM EDTA, 50% glycerol.

STORAGE CONDITIONS: Store at -20 °C.

FUNCTIONAL ASSAY: Treated PCR product with Exonuclease I to degrade unincorporated primers before performing sequencing reaction with Sequenase™ Version 2.0 DNA Polymerase Sequencing Kit (Affymetrix: PN 70170).

ASSAY CONDITIONS: The reaction mixture (100 µl) contains 67 mM glycine buffer (pH 9.5), 10 mM 2-mercaptoethanol, 6.7 mM $MgCl_2$, 0.5 mM denatured DNA, and enzyme. Incubation is at 37 °C for 30 min.

HEAT INACTIVATION: 80 °C for 15 min. Degrades to terminal dinucleotides. Degrades glycosylated DNA.

DESCRIPTION: Exonuclease I hydrolyzes single-stranded DNA in the 3'→5' direction, releasing 5'-mononucleotides and leaving the terminal 5'-dinucleotide intact. Hydrolysis is processive and cannot proceed if the 3' terminus is phosphorylated. Exonuclease I can be used to measure the endonucleolytic cleavage of covalently closed circular single-stranded DNA reacted with an endonuclease of interest. In addition, DNA helicase activity can be measured utilizing Exonuclease I. Exonuclease I is particularly useful in preparing the products of PCR for applications involving sequencing or labeling methods. Typically, the excess primers and any other extraneous single-stranded DNA present in PCR products will interfere with subsequent enzymatic reactions involving DNA synthesis. The hydrolytic properties of Exonuclease I degrade all single-stranded DNA present in the PCR mixture allowing the product to be used more efficiently in other applications. When combined with Shrimp Alkaline Phosphatase for dNTP dephosphorylation, the use of alternative purification methods, such as columns, gels or magnetic separations, are completely eliminated.

Exonuclease Comparison

Exonuclease	Gene(s)	DNA Substrates	Mode of Action	Acid-Soluble Products
Exonuclease I	*sbcB(xonA)*	Single-stranded	3'→5'	5'-dNMP 5'-terminal pNpN
Exonuclease III	*xthA*	Duplex	3'→5'	5'-dNMP
Exonuclease V	*recB, xseC*	Single-stranded and duplex	3'→5' 5'→3'	Oligonucleotides
Exonuclease V, RecBCD	*recB, recC, recD*	Single-stranded and duplex	3'→5' 5'→3'	Oligonucleotides
Exonuclease VII	*xseA, xseB*	Single-stranded	3'→5' 5'→3'	Oligonucleotides
Lambda Exonuclease	*λ, red α*	Duplex 5' Phosphorylated Band	5'→3'	5'-dNMP
T7 Gene 6 Exonuclease	*T7 gene 6*	Duplex	5'→3'	Oligonucleotides 5'-dNMP

APPLICATIONS:

1. Elimination of residual single-stranded DNA containing a 3' terminus.
2. Measuring endonucleolytic cleavage of covalently closed circular ssDNA.
3. Measuring DNA helicase activity.

RELATED PRODUCTS:

- **rSAP (Cat. No. 9216 (250 UN))**
- ***E. coli* Exonuclease I (M1222)**
- **DNA Polymerase I, Large (Klenow) Fragment (P1597)**