S1 Nuclease

Instruction for Use

Cat. No./Spec.: E1017-A/10,000 U

Concentration: 100 U/μL

Product Description

S1 nuclease can degrade single-stranded nucleic acids, releasing 5'-phosphorylated mononucleotides or oligonucleotides. Its activity on DNA is five times that on RNA. S1 nuclease can also cleave double-stranded DNA (dsDNA) at single-stranded regions caused by nicks, gaps, mismatches, or loops. S1 nuclease exhibits 3'-phosphomonoesterase activity.

This enzyme is a glycoprotein with a carbohydrate content of 18%.

Components

Component	*E1017-A*
S1 Nuclease (100 U/μL)	*100 μL*
5X S1 Nuclease Buffer	*1 mL × 2*

Storage Condition & Shelf Life

Store at -20°C.

Source

Aspergillus oryzae strain.

Unit Definition

A unit is defined as the amount of enzyme required to produce 1 μg of acid-soluble deoxyribonucleotides in 1 minute at 37° C.

Enzyme activity is determined in the following mixture: 30 mM sodium acetate (pH 4.5), 50 mM NaCl, 0.1 mM $ZnCl_2$, 5% (v/v) glycerol, and 800 μg/mL heat-denatured calf thymus DNA.

Scope of Application

- Removal of single-stranded overhangs from DNA fragments

- Localization of S1 transcripts

- Cleavage of hairpin loops

- In conjunction with Exonuclease III, creating unidirectional deletions within DNA fragments

Inhibition and Inactivation

- Inhibitors: metal chelating agents, PPi (inorganic pyrophosphate), Pi (inorganic phosphate), 5'-ribonucleotides, and deoxyribonucleotides.

- Inactivate by heating at 70°C for 10 minutes in the presence of EDTA.

Protocol

Removal of 3' and 5' overhangs

S1 Nuclease is capable of removing 3' and 5' single-stranded DNA overhangs as well as hairpin loops. The activity of S1 Nuclease is substrate-dependent, and it is essential to determine the best amount of enzyme and DNA through experimentation to ensure successful blunt-ending.

1. Prepare the reaction mixture:

Component	*Amount*
DNA	*~1 μg*
5X S1 Nuclease Buffer	*6 μL*
S1 Nuclease (100 U/μL)	*0.1 μL*
ddH_2O	*To 30 μL*

2. Incubate at room temperature for 30 minutes.

3. Terminate the reaction by adding 2 μL of 0.5 M EDTA and heating at 70°C for 10 minutes.

Notes

1. S1 Nuclease can be diluted immediately before use with 1X reaction buffer.

2. Under conditions of high enzyme and low salt concentrations, S1 Nuclease can

introduce breaks into double-stranded DNA, RNA, and DNA/RNA hybrids.

This product is for research use only.