S1 Nuclease

Catalog Number EN0321

Pub. No. MAN0013722 **Rev.** B00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

Contents	Amount	Storage
S1 Nuclease	10000 U, 100 U/µL	-25 °C to -15 °C
5X Reaction Buffer	6 x 1 mL	

Description

S1 Nuclease degrades single-stranded nucleic acids, releasing 5'-phosphoryl mono- or oligonucleotides. It is five times more active on DNA than on RNA (1).
S1 Nuclease also cleaves dsDNA at the single-stranded region caused by a nick, gap, mismatch or loop.
S1 Nuclease exhibits 3'-phosphomonoesterase activity. The enzyme is a glycoprotein with a carbohydrate content of 18 %.

Applications

- Removal of single-stranded overhangs of DNA fragments (2).
- S1 transcript mapping (3, 4).
- Cleavage of hairpin loops.
- Creation of unidirectional deletions in DNA fragments in conjunction with Exo III (5).

Source

Aspergillus oryzae cells.

Definition of Activity Unit

One unit of the enzyme produces 1 µg of acid soluble deoxyribonucleotides in 1 min at 37 °C.
Enzyme activity is assayed in the following mixture: 30 mM sodium-acetate (pH 4.5), 50 mM NaCl, 0.1 mM $ZnCl_2$, 5 % (v/v) glycerol, 800 µg/mL heat denatured calf thymus DNA.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 mM $ZnCl_2$ and 50 % (v/v) glycerol.

5X Reaction Buffer

200 mM sodium acetate (pH 4.5 at 25 °C), 1.5 M NaCl and 10 mM $ZnSO_4$.

Inhibition and Inactivation

- Inhibitors: metal chelators, PP_i, P_i, 5'-ribonucleotides and deoxyribonucleotides.
- Inactivated by heating at 70 °C for 10 min in the presence of EDTA.

Note

S1 Nuclease can introduce breaks into double-stranded DNA, RNA and DNA/RNA hybrids at high enzyme and low salt concentrations (6).

Protocol for Removal of 3'- and 5'-overhangs with S1 Nuclease

S1 Nuclease removes 3' and 5' single stranded DNA overhangs and hairpin loops. The activity of S1 Nuclease is substrate-dependent and the optimal enzyme and DNA amounts for successful blunting should be determined experimentally.

1. Prepare the following reaction mixture:

Components	Volume
DNA	~1 µg
5X Reaction Buffer for S1 Nuclease	6 µL
S1 Nuclease	0.1 µL (10 U)
Water, nuclease-free (#R0581)	to 30 µL
Total volume	**30 µL**

2. Incubate the mixture at room temperature for 30 min.
3. Stop the reaction by adding 2 µL of 0.5 M EDTA and heating at 70 °C for 10 min.

Note

The S1 Nuclease can be diluted with 1X reaction buffer immediately prior to use.

References

1. Lehman, R.I., Endonucleases specific for single-stranded polynucleotides, The Enzymes, 3rd. Ed. (Boyer, P.D., ed.), 4, 193-201, 1981.
2. Roberts T.M., et al., A general method for maximizing the expression of a cloned gene, Proc. Natl. Acad. Sci. USA, 76, 760-764, 1979.
3. Berk, A.J., Sharp, P.A., Spliced early mRNAs of simian virus 40, Proc. Natl. Acad. Sci. USA, 75, 1274-1278, 1978.
4. Weidle, U., Weissmann, C., The 5'-flanking region of a human IFN-alpha gene mediates viral induction of transcription, Nature, 303, 442-446, 1983.
5. Henikoff, S., Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing, Gene, 28, 351-359, 1984.
6. Vogt, V.M., Purification and further properties of single-strand-specific nuclease from *Aspergillus oryzae*, Eur. J. Biochem., 33, 192-200, 1973.

Revision history: Pub. No. MAN0013722

Revision	Date	Description
B00	2025-04-28	New design template, updated buffer quantity, removed COA part

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