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ProductInformation

Deoxyribonuclease I

Catalog Number **D7291**

Storage Temperature –20 °C

TECHNICAL BULLETIN

CAS RN: 9003-98-9

EC 3.1.21.1

Synonym: DNase I

Product Description
Deoxyribonuclease I is isolated from bovine pancreas and is processed to reduce RNase activity to below detectable levels.

DNase I digests single- and double-stranded DNA to a mixture of mono- and oligonucleotides carrying 5'-phosphates and 3'-OH termini. This catalytic activity is divalent ion-dependent. In the presence of Mg^{2+}, DNase I hydrolyzes each strand of double-stranded DNA randomly and independently. In the presence of Mn^{2+}, both strands can be cleaved. It is useful for nick translation, DNase footprinting, bisulfite-mediated mutagenesis, and RNA purification[1,2], and is suitable for all but the most stringent RNA purification procedures.

However, RNA treated with this DNase I should not be used to generate a cDNA library or used for RT-PCR. For these sensitive applications, it is recommended to use DNase I**,** Amplification Grade , Catalog Number AMP-D1.

Deoxyribonuclease I is supplied as a solution in 50% (v/v) glycerol, 20 mM sodium acetate, pH 6.5, 5 mM $CaCl_2$, 0.1 mM PMSF.

Specific activity: ≥10,000 units/mg protein

Unit Definition: One unit will cause a ΔA_{260} of 0.001 per minute per ml reaction mixture using calf thymus DNA as substrate.

RNase: none detected

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store at –20 °C

Procedure
Typical protocol to remove trace DNA

Add to an RNase-free PCR tube:
- 1 µg of RNA sample in 8 µl water
- 1 µl of 10x reaction buffer (200 mM Tris-HCl, pH 8.3, with 20 mM $MgCl_2$)
- 1 µl of DNase I, 1 unit/µl*

*Refer to the Certificate of Analysis for the lot specific specific activity and the number of mg protein per ml.

Incubate for 15 minutes at room temperature

To stop add 1 µl of Stop Solution to bind calcium and magnesium ions and to inactivate the DNase I.

The Stop Solution (50 mM EDTA) must be added before heating to prevent metal (Mg/Ca) ion catalyzed hydrolysis of the RNA. Heat at 70 °C for 10 minutes to denature both the DNase I and the RNA.

Note: This product should not be used for digestions longer than 15 minutes or for digestions at temperatures higher than room temperature, or the residual contaminating RNase activity will begin to degrade the RNA.

Product Profile

Activity Assay

Assay Buffer: 100 mM sodium acetate, pH 5.0 at 25 °C, containing 5 mM $MgCl_2$
50 µg/ml calf thymus DNA

5-20 µg of DNase I was added to 3 ml of reaction mixture at 25 °C and the ΔA_{260} is monitored for 10 minutes. The maximum linear rate was used to calculate the activity.

RNase Assay

Two µg transfer RNA were incubated with 2 µg DNase I in a 50 µl reaction mixture containing 30 mM Tris-HCl, pH 7.8, 50 mM NaCl and 10 mM $MgCl_2$ for 16 hours at 37 °C. No degradation of the tRNA was detected by polyacrylamide gel electrophoresis

References

1. Galas, D.J., and Schmitz, A., DNAse footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res., **9,** 3157-3170 (1978).
2. Greenfield, L., et al., Conversion of closed circular DNA molecules to single-nicked molecules by digestion with DNAase I in the presence of ethidium bromide. Biochim. Biophys. Acta, **407,** 365-375 (1975)

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