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ProductInformation

Deoxyribonuclease I

Product No. **D 7291**

Storage Temperature –20 C

TECHNICAL BULLETIN

EC 3.1.21.1

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

Deoxyribonuclease 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.

Deoxyribonuclease I is useful for nick translation, DNase footprinting, bisulfite-mediated mutagenesis, and RNA purification.[1,2]

Reagents
Deoxyribonuclease I is supplied as a solution in 20 mM sodium acetate, pH 6.5, 5 mM $CaCl_2$, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 50 % glycerol

Precautions and Disclaimer
Deoxyribonuclease I is for R&D use only, not for drug, household, or other purposes. Please consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability
Store Deoxyribonuclease I at –20 C.

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

Procedure
Typical protocol to remove trace RNase.
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

Incubate for 15 minutes to 1 hour at room temperature (some prefer 37 °C, either is fine). To stop add 1 µl of Stop Solution to bind calcium and magnesium ions and to inactivate the DNase I.

Note: 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.

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

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

RNase Assay
Two micrograms of transfer RNA were incubated with 2 micrograms 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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