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RNase-Free DNase I

Cat. Nos. D9902K, D9905K, and D9910K

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1. Introduction

RNase-Free DNase I is an endonuclease that efficiently hydrolyzes double- (ds) or single-stranded DNA to a mixture of short oligo- and mononucleotides. In the presence of Mg^{2+}, cleavage of each strand of a dsDNA substrate proceeds independently.[1] In contrast, in the presence of Mn^{2+}, the enzyme cleaves both strands of DNA at approximately the same site to generate molecules with blunt ends or 1- or 2-base overhangs[1] that can be blunted with T4 DNA Polymerase.

Provided at a concentration of 1 MBU/µl, RNase-Free DNase I is available in 2,500-, 5,000-, and 10,000-MBU sizes and includes 10X Reaction Buffer.

2. Applications

- Elimination of the DNA template following *in vitro* RNA synthesis with T7 Phage RNA Polymerase.
- Characterization of DNA:protein interactions by of "DNase I footprinting".[1,2]
- Treatment of RNA prior to RT-PCR.[3]
- Radiolabeling of DNA by nick translation.[1,4]

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 50 mM $CaCl_2$, and 10 mM $MgCl_2$.

DNase I 10X Reaction Buffer: 100 mM Tris HCl (pH 7.5), 25 mM $MgCl_2$, and 5 mM $CaCl_2$.

Unit Definition: One Molecular Biology Unit (MBU) of RNase-Free DNase I produces an increase in the A_{260} of a solution of dsDNA, of 0.001 per minute at 25°C. Functionally, 1 MBU completely digests 1 µg of pUC19 DNA to oligonucleotides in 10 minutes at 37°C.

Quality Control: RNase-Free DNase I is function-tested in two assay systems. A hyperchromicity assay is performed in a reaction containing 50 µg/ml native calf thymus DNA, 0.1 M sodium acetate (pH 5.0), 5 mM $MgCl_2$, 2 mM $CaCl_2$, and varying amounts of enzyme. A digestion assay is performed in a reaction containing 33 mM Tris-acetate (pH 7.5), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM dithiothreitol, 2 mM $CaCl_2$, 1.0 µg of pUC19 DNA, and varying amounts of enzyme.

Contaminating Activity Assays: RNase-Free DNase I is free of detectable RNase activities as assayed by PAGE analysis of 1 µg of a synthetic RNA transcript following incubation with enough RNase-Free DNase I to completely digest 1 mg of DNA.

4. Example Protocol

1. Dilute DNase I 10X Reaction Buffer to 1X using RNase-Free water.
2. Prepare 50 µl of a working DNase I Solution for each sample to be treated by adding 5 µl of RNase-Free DNase I to 45 µl of 1X Reaction Buffer (from Step 1).
3. Completely resuspend 5 µg of a nucleic acid pellet in 50 µl of working DNase I solution.
4. Incubate at 37°C for 10 minutes.

5. References

1. Sambrook, J. *et al.*, (1989) in: *Molecular Cloning: A Laboratory Manual (2nd ed.)*, Cold Spring Harbor Laboratory Press, New York.
2. Galas, D.J. and Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157.
3. Kienzle, N. *et al.*, (1996) *BioTechniques* **20**, 612.
4. Rigby, P.W.J. *et al.*, (1977) *J. Mol. Biol.* **113**, 237.

6. Related Products

Cat. #	Concentration	Quantity
Plasmid-Safe™ ATP-Dependent DNase		
E3101K	10 U/µl	1,000 Units
E3105K	10 U/µl	5,000 Units
E3110K	10 U/µl	10,000 Units
Exonuclease I, *E. coli*		
X40501K	20 U/µl	1,000 Units
X40505K	20 U/µl	5,000 Units
X40520K	20 U/µl	20,000 Units
Exonuclease III, *E. coli*		
EX4405K	200 U/µl	5,000 Units
EX4425K	200 U/µl	25,000 Units
Mung Bean Nuclease		
M8202K	50 U/µl	2,000 Units
M8205K	50 U/µl	5,000 Units
OmniCleave™ Endonuclease		
OC7810K	200 U/µl	10,000 Units
OC7850K	200 U/µl	50,000 Units

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