Manual

RNase-Free DNase I manual

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RNase-Free DNase I is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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Contents

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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.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
RNase-Free DNase I	5,000 MBU	D9905K	DNase I, RNase-Free (1 U/µL)	E0013-1D4	5 mL
			10X DNase I Reaction Buffer	SS000751-D2	5.5 mL
	10,000 MBU	D9910K	DNase I, RNase-Free (1 U/µL)	E0013-1D4	10 mL
			10X DNase I Reaction Buffer	SS000751-D2	11 mL

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; also called Unit (U)) 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 (1 U) 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. Applications

- Elimination of the DNA template following *in vitro* transcription reactions.
- Characterisation of DNA:protein interactions by “DNase I footprinting”.[1,2]
- Treatment of RNA prior to RT-PCR.[3]
- Radiolabeling of DNA by nick translation.[1,4]

5. 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 re-suspend 5 µg of a nucleic acid pellet in 50 µL of working DNase I solution.
4. Incubate at 37 °C for 10 minutes.

6. 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.

7. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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