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RNase I

Cat. No. N6901K

1. Introduction

RNase I preferentially degrades single-stranded RNA to individual nucleoside 3′ monophosphates by cleaving every phosphodiester bond.[1] By comparison, other ribonucleases cleave only after specific residues (e.g., RNase A cleaves 3′ to pyrimidine residues). Thus, RNase I is useful for removing RNA from DNA preparations,[2] detecting mismatches in RNA:RNA and RNA:DNA hybrids[2,3], and analyzing and quantifying RNA in ribonuclease protection assays (RPA).[4,5] The enzyme is completely inactivated by heating at 70°C for 20 minutes in the presence of 5 mM dithiothreitol (DTT), eliminating the requirement to remove the enzyme prior to many subsequent procedures.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
RNase I, *E. coli*	1,000 Units	N6901K	RNase I (10 U/µL)	E0067-10D1	100 µL
			DTT (0.1 M)	SS000065-D1	2.5 mL
			RNase I Dilution Buffer	SS000255-D1	1 mL
			10X TNE Buffer	SS000806-D1	5 mL

3. Product Specifications

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

Storage Buffer: RNase I is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, and 0.1 mM EDTA.

RNase I Dilution Buffer: A 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, and 0.1 mM EDTA.

10X TNE Buffer: 100 mM Tris-HCl (pH 7.5), 1 M NaCl, and 10 mM EDTA.

Unit Definition: One unit degrades 100 ng of *E. coli* ribosomal RNA per second into acid-soluble nucleotides at 37°C.

Quality Control: RNase I is function-tested in a reaction containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, and 60 µg of *E. coli* ribosomal RNA with varying amounts of enzyme.[6]

Contaminating Activity Assays: RNase I is free of detectable exo- and endodeoxyribonuclease activities as judged by incubation of 1 µg of various DNA substrates with 4 x 10^6 U of enzyme at 37°C for 16 hours.

4. Protocol for Removing RNA from DNA Preparations

RNase I can be used in place of RNase A for removing RNA from DNA preparations. In contrast to RNase A, RNase I effectively degrades contaminating RNA to mono- and dinucleotides that will not interfere with visualization of small DNA molecules. After RNA removal, the enzyme can be inactivated by heating at 70°C for 20 minutes in the presence of 5 mM DTT.

Protocol

1. Isolate DNA from 1-2 mL of overnight bacterial culture using a standard alkaline lysis procedure.[5]
2. After ethanol precipitation, suspend the DNA in 1X TNE buffer (page 2) at a concentration appropriate for subsequent applications (see Notes II below).
3. Dilute RNase I ten-fold with RNase I Dilution Buffer and add 1.5-2 U to the DNA preparation.
4. Incubate at 37°C for 30 minutes to degrade contaminating RNA.
5. Add DTT to a final concentration of 5-10 mM.
6. Incubate at 70°C for 20 minutes to inactivate the enzyme.

Notes

Reaction Buffer: Incubation with RNase I can be performed simultaneously with the digestion of plasmid DNA by restriction endonucleases. RNase I maintains ≥90% activity in buffers containing between 100 mM to 200 mM salt (either NaCl or KOAc). The activity of the enzyme is also relatively constant over a pH range of 7.0-8.8. Therefore, if the restriction endonuclease buffer is within these parameters, RNase I digestion can be performed in the restriction endonuclease buffer.

Enzyme Dilution: Diluted enzyme may be stored for up to two months at –20°C in a freezer without a defrost cycle.

5. References

1. Shen, V. and Schlessinger, D. (1982) *The Enzymes **XV** (Part B)*, 501.
2. Winter, E. *et al.*, (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7575.
3. Myers, R.M. *et al.*, (1985) *Science* **230**, 1242.
4. Sambrook, J. *et al.*, (1989) in: *Molecular Cloning: A Laboratory Manual (2nd ed.)*, Cold Spring Harbor Laboratory Press, New York.
5. Saccomanno, C.F. *et al.*, (1992) *BioTechniques* **13**, 847.
6. Corbishley, T.P. *et al.*, (1984) *Meth. Enzymatic Anal.* **4**, 134.