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RNase I, *E. coli*

Cat. Nos. N6901K and N6905K

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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[2-4], and analyzing and quantifying RNA in ribonuclease protection assays (RPA).[5,6] 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 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.

Enzyme 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.[7]

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.

3. Kit Contents

Desc.	Concentration	Quantity
RNase I, from *E. coli* is available in 1,000- and 5,000- (sold as 5 x 1,000) unit sizes. The 1,000- unit size contains the following components:		
Ribonuclease I, *E. coli*	(10 U/µl)	100 µl
Ribonuclease I Dilution Buffer		1 ml
10X TNE Buffer		5 ml
100 mM Dithiothreitol		2.5 ml

4. Related Products

The following products are also available:

- AmpliScribe™ T7-*Flash*™ Transcription Kit
- RNase H, *E. coli*
- RNase R, *E. coli*
- RNase T1, *E. coli*
- RNase III

5. Suggested Protocol for Mismatch Detection

This protocol is suitable for the detection of mismatched bases in RNA:RNA or RNA:DNA hybrids.[2-4] RNA transcripts can be generated from mutant and wild-type sequences cloned in the proper orientation into vectors containing the phage RNA polymerase promoters. Alternatively, PCR products containing the appropriate phage promoter sequences as part of the amplification primers can be used as templates for the *in vitro* transcription reactions.

Protocol

1. Synthesize the necessary sense and/or anti-sense transcripts of the mutant and wild-type genes.
2. Quantify the transcripts by absorption at A_{260}.
3. Set up a set of four reaction tubes each containing 500 ng of the following transcripts:

   Tube #1: wild-type sense and anti-sense transcripts (negative control).

   Tube #2: wild-type sense and mutant anti-sense transcripts.

   Tube #3: wild-type anti-sense and mutant sense transcripts.

   Tube #4: wild-type sense and mutant anti-sense transcripts (RNase I-minus control).
4. Add 5 µl of 10X TNE buffer (1X TNE buffer = 10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 1 mM EDTA) and bring the volume to 50 µl with RNase-free water.
5. Heat the reaction tubes at 70°C for 2 minutes.
6. Cool the reaction tubes slowly to room temperature over a 15 minute period.
7. Dilute RNase I ten-fold with Enzyme Dilution Buffer and add 1 U of enzyme to each reaction tube except the enzyme-minus control (see Notes I below).
8. Incubate the reaction tubes at 37°C for 30 minutes (see Notes I below).
9. To inactivate the RNase I, add 2.5 µl of 100 mM DTT to the reaction and incubate it at 70°C for 20 min. Alternatively, stop the reaction by adding a denaturing gel loading buffer to the sample.
10. Separate the products on a denaturing agarose or acrylamide gel, and visualize by staining with ethidium bromide or other appropriate fluorescent dye.
11. Mismatches are identified as cleavage fragments. The mutation can be localized relative to the ends of the RNA by determining the size of the cleavage fragments. Using smaller RNA or DNA transcripts can help to localize the mutation more precisely.

Notes I

Enzyme Concentration and Digestion Time: It is often necessary to optimize enzyme concentration and times of digestion to ensure maximal cleavage of the mutation without causing excessive non-specific digestion of double-stranded molecules. The amount of enzyme and time of digestion listed in the protocol is suitable for initial experiments.

Base Recognition and Cleavage: Despite the ability to cleave after each nucleotide, RNase I digestion will not identify all mutations with equal efficiency.[2,3] This suggests that nucleotides surrounding the mismatch, or secondary structure of the hybrid, can affect which mismatches are recognized and cleaved by RNase I.[3]

6. Protocol for Removing RNA from DNA Preparations

RNase I can be used in place of RNase A for removing RNA from DNA preparations.[2] 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.[6]
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 Enzyme 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 II

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.

7. References

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2. Johnson, M. (1996) *Epicentre Forum* **3** (4), 7.
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