



RNase I

Product	Quantity	Cat. No.	Remarks
RNase I	10,000 unit	EBT-3021	100 unit/ μ l

Description

RNase I Ribonuclease catalyzes the degradation of RNA to cyclic nucleotide monophosphate intermediates. RNase I Ribonuclease is one of the few known RNases that can cleave a phosphodiester bond between any two ribonucleotides. RNase I Ribonuclease may be used 1) to remove RNA from DNA preparations, 2) for RNase protection assays and 3) for mapping or quantitation of RNA by selective cleavage of single-stranded regions. RNase I is purified from a recombinant *E.coli* strain.

Concentration & Storage Condition

100 unit/ μ l. Store at -20 $^{\circ}$ C.

Storage Buffer

10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 50% glycerol.

10x Reaction Buffer

100 mM Tris-HCl, pH 7.5, 50 mM EDTA, 2 M sodium acetate. .

Unit Definition

One unit is defined as the amount of enzyme required to completely degrade RNA at the rate of 100 ng/sec at 37 $^{\circ}$ C in 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 200 mM sodium acetate, 0.2 μ g RNA, 0.05% NP-40 and 2 μ g BSA.

QC Tests

Activity, exo and endonuclease activity test, SDS-PAGE purity, performance tests.

Usage Information

Removing RNA from Purified DNA

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.

Procedure

1. Purified DNA from 1-2 ml of overnight bacterial culture using a alkaline lysis method.
2. After ethanol precipitation, suspend the DNA in 1x Reaction Buffer
3. Dilute RNase I ten-fold with Enzyme Dilution Buffer and add 1.5-2 unit to the DNA preparation.
4. Incubate at 37 $^{\circ}$ C for 30 min to degrade contaminating RNA.
5. Incubate at 70 $^{\circ}$ C for 15 min 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 to 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 $^{\circ}$ C in a freezer.