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Ribonuclease T1, *Aspergillus oryzae*

Cat. Nos. NT09100K and NT09500K

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1. Introduction

Ribonuclease T1 (RNase T1, E.C. 3.1.27.3) is an endoribonuclease that specifically cuts RNA or deaminated RNA at the 3′ end of guanosine residues and adjacent nucleotides through a 2′,3′-cyclic phosphate intermediate mechanism. Unlike RNase A which degrades RNA through cleavage at pyrimidine residues, or RNase I which cleaves at many sites along the RNA molecule, RNase T1 specifically cuts RNA molecules at guanine residues, making it suitable for assays where base-specific cleavage is desirable, (e.g., RNA mapping, RNA structure studies and RNA sequencing, due to its cleavage specificity,[1-3] or as a general RNase in extraction and purification of DNA from mammalian cells[4]). Epicentre's RNase T1 is cloned from *Aspergillus oryzae* and over expressed in *E. coli* to produce a highly pure enzyme without contaminating nuclease activities. The enzyme is active in a broad range of reaction buffers.

2. Product Specifications

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

Storage Buffer: RNase T1 is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 0.1 mM EDTA.

Unit Definition: One unit converts 100 ng of *E. coli* ribosomal RNA into acid-soluble nucleotides in 5 seconds at 37°C.

Quality Control: RNase T1 is function-tested in a 100-µl reaction containing: 120 µg *E. coli* ribosomal RNA in 50 mM Tris-HCl (pH 7.0) and 2 mM EDTA for 10 minutes at 37°C.

Contaminating Activity Assays: RNase T1 is free of detectable DNA exo- and endonuclease activities, and non-specific RNase activities.

Reaction Buffer: RNase T1 treatment can be performed simultaneously with the digestion of plasmid DNA by restriction endonucleases. RNase T1 maintains >90% of its activity in buffers containing 100-200 mM salt (NaCl or KOAc). The activity of RNase T1 is also relatively constant over a pH range of 7.0-8.8. Therefore, if the restriction endonuclease buffer is within these parameters, digestion of contaminating RNA using RNase T1 can be performed in the restriction endonuclease buffer.

Since divalent cations (Mn^{2+}, Cu^{2+}, Zn^{2+}) inhibit the activity of RNase T1, EDTA should be included in the reaction buffer. RNase T1 activity can be stopped by the addition of 1/10 volume 10% SDS followed by phenol extraction.

Dilution Buffer: RNase T1 may be diluted to a lower working concentration in the indicated Storage Buffer.

3. Kit contents

Cat. #	Quantity
Ribonuclease T1 is available in two sizes:	
NT09100K	100,000 Units
NT09500K	500,000 Units

Each is supplied at a concentration of 1,000 Units/µl.

4. Related Products

The following products are also available:

- RiboShredder™ RNase Blend
- RNase I
- RNase III
- OmniCleave™ Endonuclease
- MasterPure™ DNA Purification Kit for Blood
- MasterPure™ Complete DNA and RNA Purification Kit

5. References

1. Wang, L. *et al.*, (1976) *Proc. Nat. Acad. Sci. USA* **73** (1), 3952.
2. Donis-Keller, H. *et al.*, (1977) *Nucl. Acids Res.* **4** (8), 2527.
3. Frisby, D., *et al.*, (1977) *Nucl. Acids Res.* **4** (9), 2975.
4. Rubsam, L.Z. *et al.*, (1997) *J. Chromatogr. B. Biomed. Sci. Appl.* **702** (1-2), 61.

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