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Ribonuclease R, *E. coli*

Cat. No. RNR07250

1. Introduction

Ribonuclease R (RNase R) from *E. coli*, is a magnesium-dependent 3′→5′ exoribonuclease that digests essentially all linear RNAs but does not digest lariat or circular RNA structures, or double-stranded RNA with 3′-overhangs shorter than 7 nucleotides.[1,2] Most cellular RNAs will be digested completely by RNase R, with the exception of tRNAs, 5S RNA and intron lariats. The 3′-tails of lariats will be trimmed by RNase R to the branch point nucleotide, where there is a 2′,5′-phosphodiester linkage.

Lariats are produced during pre-mRNA splicing of intron regions and can be isolated from a mixture of total RNA by digestion with RNase R. The MasterPure™ Complete DNA and RNA Purification Kit and MasterPure™ Yeast RNA Purification Kits are ideal for such total RNA preparations. RNA isolated in this way can be used as a template to produce labeled cDNA which is then used as a target for microarrays containing potential intron sequences or for tiling arrays containing overlapping regions of complete chromosomes or genomes. The cDNA produced in this way will not be a linear representation of the intron, but the sequences contained in it will be intron-derived.

RNase R is provided as a 250 U size (20 U/µL; 1 µg/µL) and is supplied with a 10X RNase R Reaction Buffer.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
RNase R	250 Units	RNR07250	Ribonuclease R (20 U/µL)	E0111-20D1	12.5 µL
			10X RNase R Reaction Buffer	SS000769-D1	250 µL

3. Product Specifications

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

Storage Buffer: RNase R is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton® X-100 and 1 mM dithiothreitol.

Unit Definition: One unit converts 1 µg of poly-r(A) into acid-soluble nucleotides in 10 minutes at 37°C in 20 mM Tris-HCl (pH 8.0), 100 mM KCl and 0.1 mM $MgCl_2$.

10X RNase R Reaction Buffer: is: 0.2 M Tris-HCl (pH 8.0), 1 M KCl and 1 mM $MgCl_2$.

Note: *RNase R requires low (0.1-1.0 mM) magnesium concentrations for activity. Low EDTA concentrations in substrate RNA solutions can negatively affect RNase R activity. Additional $MgCl_2$, up to 1 mM final concentration can be used to compensate for EDTA in the substrate. Optimal activity is at 37°C.*

Quality Control: RNase R is function-tested in a reaction containing a mixture of linear and circularized RNA oligonucleotides. Only the linear RNA is digested.

Contaminating Activity Assays: RNase R is free of detectable endoribonuclease and DNase activities.

4. Applications

- Alternative splicing studies
- Gene expression studies
- Intron cDNA production
- Intronic screening of cDNA libraries
- Isolation of splicing intermediates and lariats

5. References

1. Suzuki, H. *et al.*, (2006) *Nucl. Acids Res.* **34** (8) e63.
2. Vincent, H.A. and Deutscher, M.P., (2006) *J. Biol. Chem.* **281** (40) 29769.

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