Manual

# Ribonuclease R, *E. coli*

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Ribonuclease R, *E. coli* is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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# Manual

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## 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 labelled 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 | Catalogue number | Reagent description | Part number | 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 (Rohm & Haas) 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 circularised 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 HA and Deutscher MP (2006) *J. Biol. Chem.* 281 (40) 29769.

## 6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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