CELLSCRIPT™
RNA for Translation in Cells

# RNase R

Cat. No. **RRT250325**

## INTRODUCTION

**Ribonuclease R** (RNase R) from *E. coli*, is a 3'→5' exoribonuclease that can digest linear RNA and Y-structure RNA, but does not digest circular RNA (circRNA), lariat intron RNA, blunt-ended double-stranded RNA (dsRNA), dsRNA with 3'-overhangs <7 nt in length or single-stranded DNA.[1,2] Highly structured linear cellular RNAs such as prokaryotic 23S and 16S ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), transfer RNAs (tRNAs) and histone mRNAs are also not efficiently degraded by RNase R.[3] This functionality makes RNase R ideal for isolating circular RNA structures from total cellular RNA preparations for use in intron and splicing studies and natural cellular circRNA identification. Additionally, RNase R is used as a crucial component in *in vitro* circRNA preparation cleanup and enrichment[3-5] with both RNA Ligase-based[6] or self-splicing permutated intron–exon sequence (PIE method)-based[5,7] methodologies.

## MATERIALS

### Materials Supplied

**Important** Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

| RNase R | |
|---|---|
| Component | Volume |
| RNase R, 1 µg/µl<br>in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA and 0.1% Triton® X-100. | 25 µl |
| 10X RNase R Reaction Buffer<br>0.2 M Tris-HCl, pH 8.0, 1 M KCl and 1 mM $MgCl_2$. | 50 µl |

## SPECIFICATIONS

### Unit Definition

RNase R is provided using mass concentration as opposed to units concentration. Because poly(rA) is the preferred substrate for RNase R, the traditionally-reported RNase R units based on poly(A) digestion quantities do not accurately reflect the digestion rate on linear RNAs comprised of mixed nucleosides.

### Functional Testing

RNase R is functionally 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 DNase and non-RNase R RNase activities.

For more information, consult the appropriate safety data sheet (SDS) at www.cellscript.com.



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## BEFORE YOU START: IMPORTANT TIPS FOR RNASE R DIGESTION

- **RNA Source**:

  The RNA to be treated should be purified and resuspended in RNase-Free Water prior to RNase R treatment. **Do not resuspend the RNA in an EDTA-containing solution**.

- **Inclusion of RNase Inhibitor**:

  We recommend the addition of ScriptGuard™ RNase Inhibitor (sold separately) to RNase R treatment reactions. ScriptGuard RNase Inhibitor inhibits RNases A, B & C, thus protecting your RNA from degradation by RNase contamination, but does not inhibit RNase R activity.

- **Maintaining an RNase-Free Environment**:

  Highly stable RNases are ubiquitous, including on human skin.

  Creating an RNase-free work environment and maintaining RNase-free solutions is critical for successful work with RNA.

  We strongly recommend to:

  – Use RNase-free tubes and pipette tips.

  – Always wear gloves when handling kit components or samples containing RNA and change gloves frequently, especially after touching potential sources of RNase contamination such as doorknobs, pens, pencils and human skin. Do not touch any kit component or tube containing RNA with an ungloved hand.

  – Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

**Example Data**:

**Figure 1** PIE method RNA circularization prep untreated (UT) and treated (T) with RNase R. Formaldehyde-denaturing 2% agarose gel. mw) ssRNA molecular weight markers

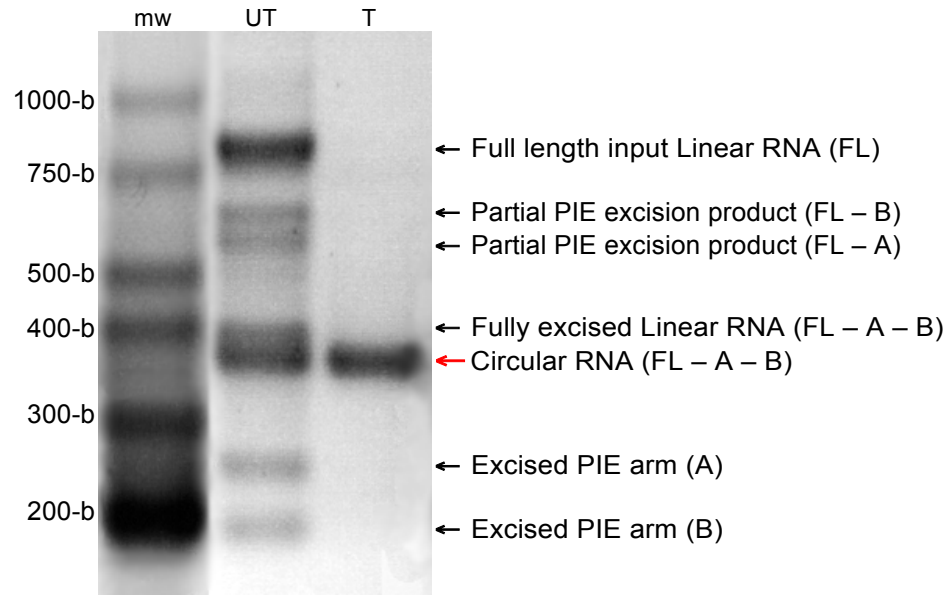
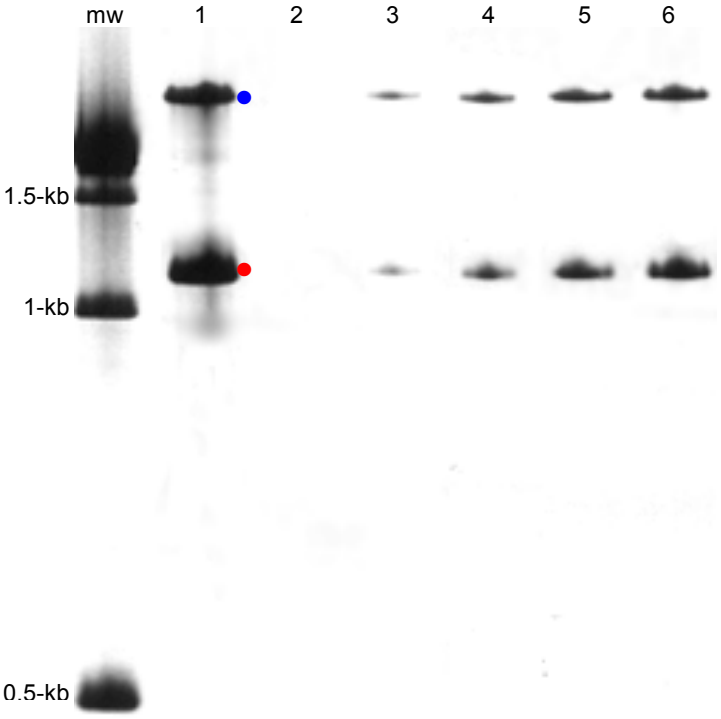


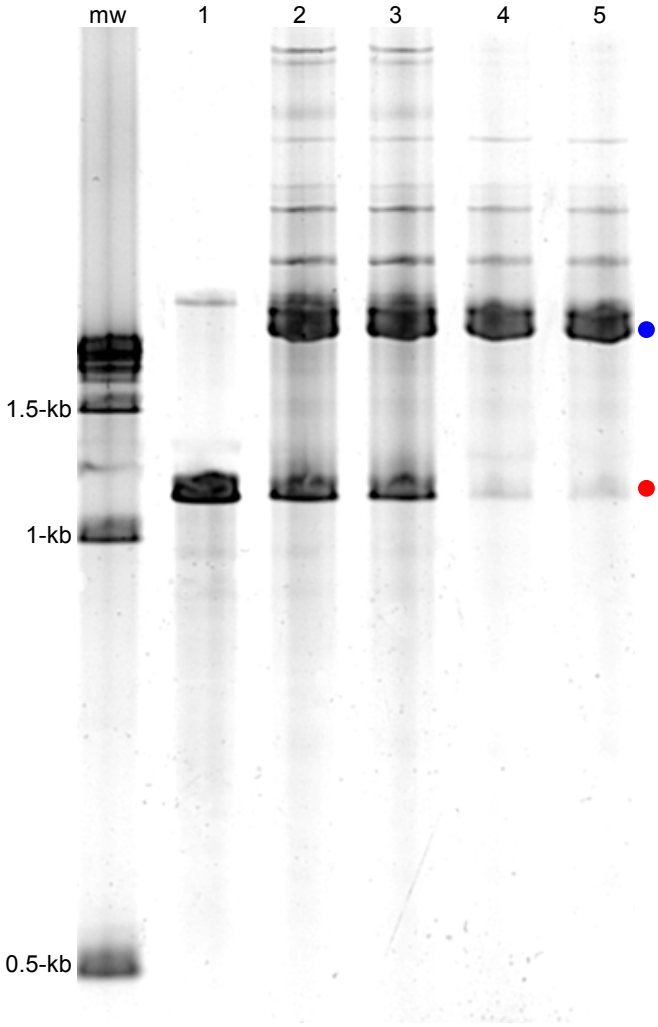

**Figure 2** 5 μg of linear RNA untreated and treated with varying amounts of RNase R (37°C, 30 minutes). 8 M Urea, 4% polyacrylamide gel.




mw) ssRNA molecular weight markers

1) Non-RNase R-treated input linear RNA
   ● = linear unit length, ● = linear aggregated
2) Treated with 1 μg RNase R
   (5 μg RNA / 1 μg RNase R)
3) Treated with 0.5 μg RNase R
   (10 μg RNA / 1 μg RNase R)
4) Treated with 0.25 μg RNase R
   (20 μg RNA / 1 μg RNase R)
5) Treated with 125 ng RNase R
   (40 μg RNA / 1 μg RNase R)
6) Treated with 62.5 ng RNase R
   (80 μg RNA / 1 μg RNase R)

**Figure 3** RNA Ligase method RNA circularization prep untreated and treated with RNase R.
5 μg of linear input RNA was circularized with RNA Ligase and treated or untreated with 1 μg RNase R at 37°C for 30 & 60 minutes.
8 M Urea, 4% polyacrylamide gel.




mw) ssRNA molecular weight markers

1) Input linear RNA, non-RNA Ligase treated, non-RNase R treated.

2) RNA Ligase treated, RNase R untreated for 30 min.

3) RNA Ligase treated, RNase R untreated for 60 min.

4) RNA Ligase treated, RNase R treated for 30 min.

5) RNA Ligase treated, RNase R treated for 60 min.

● = Circular RNA

● = Linear RNA

## PROCEDURE

### A. RNase R Treatment

1. Because poly(rA) is the preferred substrate for RNase R, traditionally-reported RNase R units based on poly(A) digestion quantities do not accurately reflect the digestion rate on linear RNAs comprised of mixed nucleosides. The standard reaction presented below will digest 5 μg of linear mixed nucleoside-containing RNA, thus total input RNA (linear + circular RNA) into the reaction could be >5 μg as long as the anticipated linear RNA content is ≤5 μg, as would be expected in the final reaction product of an RNA circularization reaction. When in doubt, use only 5 μg of total RNA as input into the reaction.

   **Note:** Different RNAs are digested at different rates based on RNA 3' end availability and secondary structure content of the substrate RNA. Amounts of RNase R required for a complete digestion of some RNAs may need to be optimized if the standard reaction conditions do not result in complete digestion of the linear input RNA.

   Combine the following reagents:

| **Standard RNase R Reaction** (step 1) | |
|---|---|
| Component | Amount |
| RNase-Free Water | x μl |
| 10X RNase R Reaction Buffer | 2 μl |
| ScriptGuard RNase Inhibitor (sold separately) | 0.5 μl |
| 5 μg RNA | y μl |
| Total Volume | 19 μl |

The use of ScriptGuard RNase Inhibitor is optional.

2. Mix gently and briefly centrifuge to collect the reagents in the bottom of the tube.

| **Standard RNase R Reaction** (step 2) | |
|---|---|
| Component | Amount |
| Cocktailed reaction components (from step 1) | 19 μl |
| RNase R (1 μg) | 1 μl |
| Total Reaction Volume | 20 μl |

3. Incubate at 37°C for 30 minutes.

4. Stop the reaction using any one of the following methods:
   a) immediate storage at –20°C or –70°C.
   b) Addition of EDTA to a final concentration of >2 mM.
   c) Phenol/chloroform extract then precipitate using salt/alcohol.
   d) Or other preferred method. See RNA Purification (page 6).

**B. RNA Purification**

Purify the RNA by your preferred method. The method chosen should remove residual proteins, unincorporated NTPs from the RNA and RNase R reaction products. Several options are listed below. RNA can be stored at –20°C or –70°C. If the RNA is to be stored indefinitely, store the RNA as an ethanol pellet.

I) **RNA-Binding Purification Column**: Several options are available commercially from multiple vendors. Follow the manufacturer's recommended protocol. The final resuspension of RNA should be in RNase-Free Water, TE or other suitable buffer.

II) **RNAClean XP beads** (Beckman Coulter): Removes all proteins, unincorporated NTPs and RNase R digestion products from the RNA. Follow the manufacturer's recommended protocol. The final resuspension of RNA should be in RNase-Free Water, TE or other suitable buffer.

III) **Lithium Chloride Precipitation**: Selectively precipitates ssRNA, while leaving most of the protein unincorporated NTPs and RNase R digestion products in the supernatant. Note: for this method, the RNA to be purified must be >100 bases in size.

1) Add one volume of 5 M lithium chloride, mix well.
2) Incubate for 15 minutes on ice.
3) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
4) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
5) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
6) Allow pellet to dry, then resuspend in RNase-Free Water, TE or other suitable buffer.
7) While usually unnecessary, steps 1-6 may be repeated a second time for even cleaner RNA.

IV) **Ammonium Acetate Precipitation**: Selectively precipitates ssRNA, while leaving most of the protein unincorporated NTPs and RNase R digestion products in the supernatant. Note: for this method, the RNA to be purified must be >100 bases in size.

1) Add one volume of 5 M ammonium acetate, mix well.
2) Incubate for 15 minutes on ice.
3) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
4) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
5) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
6) Allow pellet to dry, then resuspend in RNase-Free Water, TE or other suitable buffer.
7) While usually unnecessary, steps 1-6 may be repeated a second time for even cleaner RNA.

V) **Organic Extraction / Ammonium Acetate Precipitation**: Removes all proteins and selectively precipitates RNA, leaving most of the unincorporated NTPs and RNase R digestion products in the supernatant. Note: for this method, the RNA to be purified must be >100 bases in size.

1) Add one volume of TE-saturated phenol/chloroform. Vortex vigorously for 10 seconds.
2) Spin in a microcentrifuge at >10,000 x g for 5 minutes to separate the phases.
3) Remove the aqueous (upper) phase with a pipette and transfer to a clean tube.
4) Add one volume of 5 M ammonium acetate, mix well then incubate for 15 minutes on ice.
5) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
6) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
7) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
8) Allow pellet to dry, then resuspend in RNase-Free Water, TE or other suitable buffer.

Continued on next page.

### B. RNA Purification

VI) **Organic Extraction / Chromatography / Ethanol Precipitation**: Removes all proteins, unincorporated NTPs and RNase R digestion products from the RNA.

1) Add one volume of TE-saturated phenol/chloroform. Vortex vigorously for 10 seconds.
2) Spin in a microcentrifuge at >10,000 x g for 5 minutes to separate the phases.
3) Remove the aqueous (upper) phase with a pipette and transfer to a clean tube.
4) Remove unincorporated NTPs and RNase R digestion products by spin column chromatography. For commercially-available columns, follow the manufacturer's instructions for this step.
5) Add one-tenth volume of 3 M sodium acetate and 2.5 volumes of 95% ethanol to the tube, mix well.
6) Incubate for 15 minutes on ice.
7) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
8) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
9) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
10) Allow pellet to dry, then resuspend in RNase-Free Water, TE or other suitable buffer.

## TROUBLESHOOTING

| Symptom | Solution |
|---|---|
| **All linear RNA is not degraded** | Increase the time of incubation of the reaction. |
| | Increase the amount of RNase R used in the reaction. |
| | Decrease the amount of input RNA in the reaction. |
| | RNA contains stable secondary structure whether internally or at the 3' end.<br>• Add a short poly(A) tail onto the RNA to provide a structureless 3' end starting point for RNase R binding.[3]<br>• Note: some highly stable secondary structures are resistant to RNase R digestion. |

## RELATED PRODUCTS

– A-Plus™ Poly(A) Polymerase Tailing Kit
– INCOGNITO™ T7-FlashScribe™ Ψ-RNA Transcription Kit
– INCOGNITO™ T7-FlashScribe™ N1meΨ-RNA Transcription Kit
– INCOGNITO™ T7 Ψ-RNA Transcription Kit
– INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
– RNase H
– RNase I
– RNase T1
– ScriptGuard™ RNase Inhibitor
– T7-FlashScribe™ Transcription Kit V2
– T7-Scribe™ Standard RNA IVT Kit

## REFERENCES

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