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# RNase A (Biochemistry Grade)

**Catalog Number** AM2274

**Pub. No.** 4393904 **Rev.** B

| Contents | Quantity | Storage conditions |
|---|---|---|
| RNase A (Biochemistry Grade), 1 µg/mL | 200 µL | Store at –20°C. *Do not store in a frost-free freezer.* |
| 10X RNA Structure Buffer | 1 mL | |
| 1X Alkaline Hydrolysis Buffer | 1 mL | |
| 1X RNA Sequencing Buffer | 1 mL | |
| Precipitation/Inactivation Buffer (add 3.2 mL 100% ethanol before use) | 4.8 mL | |
| Gel Loading Buffer II | 1.4 mL | |
| Yeast RNA (10 mg/mL) | 100 µL | |

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

## Product description

RNase A (Biochemistry Grade) is isolated by an extensive series of purification steps including affinity chromatography. RNase A is an endonuclease that specifically cleaves 3′ of U and C residues. RNase A is used in rapid analysis of the physical structure of RNA, in combination with single-strand specific RNase T1 and double-strand specific RNase V1.

**Source:** Manufactured from Bovine pancreas by a USDA-approved source.

**Unit (U) definition:** One unit is the amount of RNase A required to give an increase of 0.0146 $A_{286}$ absorbance units per minute in a 1-mL volume at room temperature and is equivalent to 0.1177 Kunitz Units. Assay Conditions: 100 mM Tris-acetate (pH 6.5), 1 mM EDTA and 1 mM cyclic 2′, 3′–CMP.

**Storage buffer (*not included*):** 10 mM HEPES (pH 7.2), 20 mM NaCl, 0.1% Triton X-100, and 50% glycerol (v/v).

## General information about biochemistry-grade ribonucleases

Biochemistry-grade ribonucleases are optimized for the study of RNA structure, RNA sequencing, protein foot-printing, and boundary experiments. They are also tested for purity to ensure the absence of nonspecific nuclease or other contaminating ribonuclease activities that could introduce unexpected cleavage sites and interfere with RNA structure studies.

In addition to RNA structural analysis, biochemistry-grade ribonucleases can be used to map protein binding sites on RNAs by comparing cleavage patterns in the presence and absence of an RNA binding protein.

## Using RNase A (Biochemistry Grade)

To visualize the RNase A digestion products, the RNA should be end-labeled either at its 5′ end using a kinase reaction with [$\gamma$-$^{32}$P]ATP (KinaseMax™ Kit, Cat. no. AM1520), or at its 3′ end using an RNA ligation reaction with [$^{32}$P]pCp (T4 RNA Ligase, Cat. no. AM2140).

### RNA structure analysis

In the following procedure, each RNA is digested with three sequential 10-fold dilutions of ribonuclease, to quickly optimize conditions for distinguishing nucleotides that are structurally constrained from those that are not. Further dilution may be necessary to achieve the optimal digestion ladder.

**Note:** Before you begin, complete the Precipitation/Inactivation Buffer by adding 3.2 mL of 100% ethanol to the bottle supplied.

1. Thaw the 10X RNA Structure Buffer and end-labeled RNA at room temperature.

2. Mix 4 µL of 10X RNA Structure Buffer, 0.2–4 µg of end-labeled RNA, 4 µg of yeast RNA, and Nuclease-free Water to bring the final volume to 36 µL.

3. Distribute 9 µL aliquots of the RNA/buffer/water mixture into 4 microcentrifuge tubes numbered 1–4. To Sample 2 add 1 µL of ribonuclease. Mix thoroughly by pipetting.

4. Transfer 1 µL from Sample 2 to Sample 3. Mix thoroughly by pipetting.

5. Transfer 1 µL from Sample 3 to Sample 4. Mix thoroughly by pipetting.

6. Incubate all samples at room temperature for 15 minutes.

7. Add 20 µL of completed Inactivation/Precipitation Buffer, vortex and incubate at –20°C for 15 minutes.

8. Microcentrifuge at maximum speed for 15 minutes at 4°C, aspirate the supernatant, and wash the pellet with 70% ethanol.

9. Air dry the pellet and dissolve it in 7 µL of Gel Loading Buffer II.

10. Heat the samples at 95°C for 5 minutes, then fractionate 3 µL of each sample using a 6–20% acrylamide/7 M Urea sequencing gel.

11. Use autoradiography to assess the digestion products.

**Expected results:** Sample 1 is the end-labeled RNA before nuclease treatment, and any non-full length bands represent cleavage products already present within the RNA sample. These bands will also be present in the nuclease-treated samples and should be disregarded in your analysis. Samples 2, 3, and 4 were digested with decreasing amounts of ribonuclease. Bands evident in the samples with lower amounts of ribonuclease typically represent nucleotides that are most accessible to the ribonuclease.

### Alkaline hydrolysis

This procedure provides a "ladder" of hydrolyzed RNA fragments for gel electrophoresis. In the procedure, three hydrolysis times are used to provide RNA samples that are increasingly hydrolyzed. Select the ladder that provides the best distribution of nucleic acids over the range of lengths needed for your experiments.

1. Add 0.1–3 µg end-labeled RNA and 3 µg yeast tRNA in a volume not to exceed 5 µL.

2. Add sufficient 1X Alkaline Hydrolysis Buffer to bring the final reaction volume to 15 µL.

3. Distribute 5 µL aliquots of the RNA/buffer mixture into 3 tubes labeled 1–3.

4. Heat each sample to 95°C.

5. After 2 minutes, remove Sample 1 and place it in an ice bucket.

6. After 5 minutes, remove Sample 2 and place it in an ice bucket.

7. After 15 minutes, remove Sample 3 and place it in an ice bucket.

8. Add 10 µL of Gel Loading Buffer II to each of the 3 samples. Prepare an untreated sample by mixing 1 µL of 5′ end-labeled RNA with 8 µL of Gel Loading Buffer II.

9. Fractionate 3 µL of each sample (4 samples total) using a 6–20% acrylamide/7 M urea sequencing gel.

10. Use autoradiography to visualize the fractionated RNA products.

## Limited product warranty

**For Research Use Only. Not for use in diagnostic procedures.**

The information in this guide is subject to change without notice.



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9 October 2012