RNase T1
Biochemistry Grade

Store at –20°C.  
Do not store in a frost-free freezer.

Catalog #: AM2283
Amount: 200 μL
Source: An E. coli strain over expressing the Ribonuclease T1 gene of Aspergillus oryzae.
Unit Concentration: 1 U/μL
Unit Definition: 100 Units of RNase T1 is the amount of enzyme that yields an increase in absorption at 260 nm of 0.01428 units per min at room temperature using 60 μg/mL yeast total RNA as a substrate. One Unit measured using yeast RNA as substrate is equivalent to 1 Unit in the previous Ambion assay (25 Units of activity corresponds to a change of 0.01428 A₂₆₀ unit in 1 min at room temperature using GpA as substrate).

Additional Materials Included:
- 1 mL 10X RNA Structure Buffer
- 1 mL 1X Alkaline Hydrolysis Buffer
- 1 mL 1X RNA Sequencing Buffer
- 4.8 mL Precipitation/Inactivation Buffer (add 3.2 mL 100% ethanol before use)
- 1.4 mL Gel Loading Buffer II
- 100 μL Yeast RNA (10 mg/mL)

Storage Conditions:
Store at –20°C.  Do not store in a frost-free freezer.

Storage Buffer:
10 mM HEPES (pH 7.2), 1 mM EDTA, 0.1% Triton, and 50% glycerol (v/v).

USER INFORMATION

Product Description:
Ambion Biochemistry Grade Ribonucleases are optimized for the study of RNA structure, RNA sequencing, protein footprinting, 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.

Biochemistry Grade Ribonuclease T1 is isolated by an extensive series of purification steps that includes affinity chromatography. RNase T1 specifically cleaves single-stranded RNA after guanosine residues, producing 3’-phosphorylated ends. RNase T1 is used in rapid analysis of the physical structure of a target RNA.

Applications:
In addition to applications for 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. RNase T1 can also be used to perform boundary experiments to define the minimal RNA sequence required for selectable activities such as protein binding or catalysis. See the catalog page for this product at www.ambion.com for more information and resources.

To visualize the RNase T digestion products from an RNA, the RNA should be end-labeled, either at its 5’ end using a kinase reaction with [γ-³²P]ATP (KinaseMax™ Kit, Cat #AM1520), or at its 3’ end using an RNA ligation reaction with [³²P]pCp (T4 RNA Ligase, Cat #AM2140).
RNA Structure Analysis

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

We recommend that each RNA be digested with 3 different amounts of ribonuclease to quickly optimize conditions for distinguishing nucleotides that are structurally constrained from those that are not. We find that 10X dilutions of enzyme work well. Further dilution may be necessary to achieve the optimal digestion ladder.

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 your 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 min.
7. Add 20 μL of completed Inactivation/Precipitation Buffer, vortex and incubate at –20°C for 15 min.
8. Microcentrifuge at maximum speed for 15 min 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 min, 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 solvent.

RNA Sequencing

In this protocol, end-labeled RNA is denatured, then incubated with RNase T1 to partially digest the RNA 3' of guanosines. The 1X RNA Sequencing Buffer contains 7M Urea to help denature RNA secondary structure. The RNA must be diluted at least 5-fold in RNA Sequencing Buffer in step 1 for effective denaturation at 50°C in step 4.

1. Add 0.15–3 μg of end-labeled RNA and 3 μg of yeast RNA in no more than 10 μL.
2. Add sufficient 1X RNA Sequencing Buffer to bring the the final reaction volume to 27 μL.
3. Distribute 9 μL aliquots of the RNA/RNA Sequencing Buffer mixture into three microcentrifuge tubes labeled 1–3.
4. Heat each sample to 50°C for five minutes, then reduce to room temperature.
5. Add 1 μL of RNase T1 to Sample #2 and mix by pipetting.
6. Transfer 1 μL of RNase T1 from Sample #2 to Sample #3 and mix by pipetting.
7. Incubate all samples at room temperature for 15 min.
8. Add 20 μL of completed Inactivation/Precipitation Buffer, vortex, and incubate at –20°C for 15 min.
9. Microcentrifuge at maximum speed for 15 min at 4°C, aspirate the supernatant, wash with 70% ethanol, and air dry.
10. Add 7 μL of Gel Loading Buffer II, heat to 95°C for 5 min, then fractionate 3 μL 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 prior to nuclease treatment. Non-full length bands represent cleavage products 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 and #3 were digested with decreasing amounts of ribonuclease. Typically, at least one of the reactions will generate a ladder comprising digestion products from each of the guanosines in the RNA molecule. Further enzyme dilution may be necessary to achieve the optimal digestion ladder.
Alkaline Hydrolysis

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 min, remove Sample #1 to an ice bucket.
6. After 5 min, remove Sample #2 to an ice bucket.
7. After 15 min, remove Sample #3 to 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 5′ end-labeled RNA with 8 μL of Gel Loading Buffer II.
9. Fractionate 3 μL of each sample (4 total) using a 6–20% acrylamide/7 M urea sequencing gel.
10. Use autoradiography to visualize the fractionated RNA products.

Expected Results: The 3 treated samples should provide RNA samples that are increasingly hydrolyzed. Select the ladder that provides the best distribution of nucleic acids over the length of the RNA.

CERTIFICATE OF ANALYSIS

Product: RNase T1 Biochemistry Grade
Catalog #: AM2283
Lot #: 0812011
Enzyme Lot#: 0811005

Nonspecific Endonuclease Activity: Meets or exceeds specification when a sample is incubated for 14–16 hr with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis. Result: PASS

Exonuclease Activity: Meets or exceeds specification when a sample is incubated for 14–16 hr with 40 ng 32P-labeled Sau3A fragments of pUC19 and analyzed by PAGE. Result: PASS

Protease Activity: Meets or exceeds specification when a sample is incubated for 14–16 hr with 1 μg protease substrate and analyzed by fluorescence. Result: PASS

Functional Testing: The enzyme is functionally tested and analyzed using the protocols described in User Information. Result: PASS

Vahik Abouolian
Quality Assurance

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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