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RNase T1 (Biochemistry Grade)

Catalog Number AM2283

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Contents	Quantity	Storage conditions
RNase T1 (Biochemistry Grade), 1 U/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 T1 (Biochemistry Grade) 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.

Source: An *E. coli* strain over expressing the Ribonuclease T1 gene of *Aspergillus oryzae*.

Unit (U) 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 minute 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 assay (25 Units of activity corresponds to a change of 0.01 A_{260} unit in 1 minute at room temperature using GpA as substrate).

Storage buffer (*not included*): 10 mM HEPES (pH 7.2), 1 mM EDTA, 0.1% Triton, 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 T1 (Biochemistry Grade)

In addition to the applications described above, 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 web catalog page for this product at **www.lifetechnologies.com** for more information and resources.

To visualize the RNase T1 digestion products, the RNA should be end-labeled either at its 5′ end using a kinase reaction with [γ-^{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.

RNA sequencing

In the following procedure, 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.

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

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 minutes.
8. Add 20 µL of completed Inactivation/Precipitation Buffer, vortex, and incubate at –20°C for 15 minutes.
9. Microcentrifuge at maximum speed for 15 minutes 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 minutes, 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 and serves as a negative control. Non-full-length bands represent cleavage products present within the RNA sample. These bands will also be present in the ribonuclease-treated samples and should be disregarded in your analysis. Samples 2 and 3 were digested with two different 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

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

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