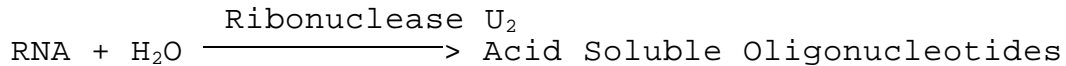


Enzymatic Assay of RIBONUCLEASE U₂
(EC 3.1.27.4)

PRINCIPLE:



Abbreviation used:

RNA = Ribonucleic acid

CONDITIONS: T = 37°C, pH 4.5, A_{260nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 200 mM Sodium Acetate Buffer, pH 4.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 37°C with 5 M HCl.)
- B. 1.2% (w/v) Ribonucleic Acid Solution (RNA)
(Prepare 3 ml in Reagent A using Ribonucleic Acid, Sigma Prod. No. R-6625.)
- C. 20 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 25 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- D. 25% (v/v) Perchloric Acid Solution (HClO₄)
(Prepare 25 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)
- E. 0.75% (w/v) Uranyl Acetate Solution (Uran Acet)
(Prepare 5 ml in Reagent D using Uranyl Acetate, Dihydrate, Fluka Stock No. 94260.)
- F. Ribonuclease U₂ Enzyme Solution
(Immediately before use, prepare a solution containing approximately 100 units/ml of Ribonuclease U₂ in cold Reagent A.)

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PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into suitable tubes:

	<u>Test</u>	<u>Blank</u>
Deionized Water	0.25	0.25
Reagent A (Buffer)	0.25	0.40
Reagent B (RNA)	0.25	0.25
Reagent C (EDTA)	0.10	0.10

Mix by swirling and equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.15	-----
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Immediately mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:

Reagent E (Uran Acet)	0.25	0.25
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Mix by swirling and allow to stand at 37°C for 15 minutes. Centrifuge for 10 minutes.

Step 2:

Pipette (in milliliters) the following reagents into suitable tubes:

Test Supernatant	0.20	-----
Blank Supernatant	-----	0.20
Deionized Water	4.80	4.80

Mix by swirling and transfer the solutions to suitable cuvettes. Record the A_{260nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(5)(1.25)(\text{df})}{(0.15)(1)(0.2)}$$

5 = Total volume (in milliliters) of assay (Step 2)
 1.25 = Volume (in milliliters) of stopped reaction (Step 1)
 df = Dilution factor

0.15 = Volume (in milliliter) of enzyme used in Step 1

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CALCULATIONS: (continued)

- 1 = The increase in A_{260nm}/minute (arbitrary value) per unit of enzyme as per the Unit Definition
0.2 = Volume (in milliliter) of stopped reaction used in Step 2

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce acid soluble oligonucleotides equivalent to a ΔA_{260nm} of 1.0 in 15 minutes at pH 4.5 at 37°C in a 1.0 ml reaction volume. Substrate: Yeast RNA

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 130 mM sodium acetate, 0.3% (w/v) ribonucleic acid, 2 mM ethylenediaminetetraacetic acid, and 15 units ribonuclease U₂.

REFERENCE:

Uchida, T. (1966) *Journal of Biochemistry* **60**, 115-132

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.