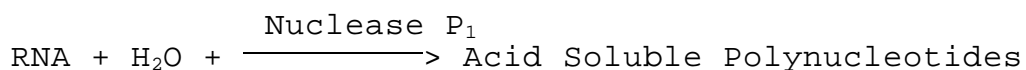


Enzymatic Assay of NUCLEASE P₁
(EC 3.1.30.1)
RNA as Substrate

PRINCIPLE:



Abbreviation used:

RNA = Ribonucleic Acid

CONDITIONS: T = 37°C, pH = 5.3, A_{260nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 0.59% (w/v) Barbital Acetate Buffer, pH 5.3 at 37°C
(Prepare 25 ml in deionized water using Barbital Buffer, Sigma Prod. No. B-6632. Adjust to pH 5.3 at 37°C with 1 M HOAc.)
- B. 0.2% (w/v) Ribonucleic Acid Solution (RNA)
(Prepare 4 ml in deionized water using Ribonucleic Acid, Sigma Prod. No. R-6625.)
- C. 2.5% (v/v) Perchloric Acid Solution (HClO₄)
(Prepare 5 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)
- D. 0.25% (w/v) Uranyl Acetate Solution (Uran Acet)
(Prepare 5 ml in Reagent C using Uranyl Acetate, Fluka Prod. No. 94260. Store on ice.)
- E. Nuclease P₁ Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Nuclease P₁ in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.20	0.20
Reagent B (RNA)	0.20	0.20

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

Reagent E (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by swirling and incubate for exactly 15 minutes at 37°C. Then add:

Reagent D (Uran Acet)	0.50	0.50
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Mix by swirling and place in an ice bath for 20 minutes. Centrifuge for 5 minutes. Remove 0.80 ml of the supernatant and dilute to 2.80 ml with deionized water. Transfer the solutions to suitable cuvettes and obtain the A_{260nm} for the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(1)(2.8)(\text{df})}{(10.6)(15)(0.1)(0.8)}$$

1 = Volume (in milliliter) of stopped reaction

2.8 = Final volume (in milliliters) of assay

df = Dilution factor

10.6 = Millimolar extinction coefficient of hydrolyzed ribonucleic acid at 260 nm

15 = Time (in minutes) of assay as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used

0.8 = Volume (in milliliter) of stopped reaction used in the

spectrophotometric determination

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

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

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UNIT DEFINITION:

One unit will liberate 1.0 μ mole of acid soluble nucleotides from RNA (R6625) per minute at pH 5.3 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.50 ml reaction mix, the final concentrations are 0.24% (w/v) barbital, 0.08% (w/v) ribonucleic acid, and 0.01 - 0.02 unit nuclease P₁.

REFERENCE:

Fujimoto, M., Kuninaka, A., and Yoshino, H. (1974)
Agricultural Biological Chemistry **38**, 777-783

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.