

**Enzymatic Assay of RIBONUCLEASE
from Chicken Liver
Polycytidylic Acid (5') as Substrate**

PRINCIPLE:

Polycytidylic Acid (5') + H₂O $\xrightarrow{\text{Ribonuclease}}$ Acid Soluble Oligonucleotides

CONDITIONS: T = 37°C, pH 6.5, A_{260nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 1 M Potassium Phosphate Monobasic Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- B. 1 M Potassium Phosphate, Dibasic, Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)
- C. 10 mM Potassium Phosphate Buffer, pH 6.5 at 37°C
(Prepare 100 ml by adjusting the pH of Reagent A with Reagent B to 6.5 at 37°C. Then dilute 1:100 with deionized water.)
- D. 0.02% (w/v) Polycytidylic Acid (5') Solution (Poly C)
(Prepare 5 ml in Reagent C using Polycytidylic Acid (5'), Potassium Salt, Sigma Prod. No. P-4903.)
- E. 200 mM Hydrochloric Acid Solution (HCl)
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- F. 20 mM Lanthanum Nitrate Solution (La(NO₃)₃)
(Prepare 50 ml in Reagent E using Lanthanum Nitrate, Hexahydrate, Sigma Prod. No. L-2388. Store on ice.)
- G. Ribonuclease Enzyme Solution
(Immediately before use, prepare a solution containing 200 units/ml of Ribonuclease in cold Reagent C.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent D (Poly C)	0.50	0.50
Equilibrate to 37°C. Then add:		
Reagent G (Enzyme Solution)	0.01	-----
Reagent C (Buffer)	-----	0.01
Immediately mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:		
Reagent F (La(NO ₃) ₃)	0.50	0.50

Store on ice for 15 minutes. Centrifuge for about 3 - 5 minutes at room temperature. Transfer 0.8 ml each of the Test and Blank supernatants to suitable cuvettes and record the A_{260nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(df)}{(1)(0.01)}$$

df = Dilution factor

1 = Extinction coefficient (arbitrary value) as per the Unit Definition

0.01 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will produce acid soluble oligonucleotides equivalent to a change in A_{260nm} of 1.0 in 15 minutes at pH 6.5 at 37°C, in a reaction volume of 1.1 ml.

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FINAL ASSAY CONCENTRATIONS:

In a 0.51 ml reaction mix, the final concentrations are 10 mM potassium phosphate, 0.02% (w/v) polycytidylic acid (5'), and 2 units ribonuclease.

REFERENCE:

Levy, C.C. and Karpetsky, T.P. (1980) *Journal of Biological Chemistry* **255**, 2153-2159

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.