

Enzymatic Assay of RIBONUCLEASE C

PRINCIPLE:

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

CONDITIONS: T = 25°C, pH 7.5, A_{260nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer with 0.05% (w/v) Bovine Serum Albumin, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 at 25°C with 1 M KOH.)
- B. 0.075% (w/v) Polycytidylic Acid (5') Solution (Poly C)
(Prepare 10 ml in Reagent A using Polycytidylic Acid (5'), Potassium Salt, Sigma Prod. No. P-4903.)
- C. 50 mM Potassium Phosphate Buffer with 2 M Potassium Chloride, pH 8.0 at 25°C (Enz Dil)
(Prepare 50 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504, and Potassium Chloride, Sigma Prod. No. P-4504. Adjust to pH 8.0 at 25°C with 1 M HCl.)
- D. 2 M Perchloric Acid Solution (HClO₄)
(Prepare 15 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)
- E. Ribonuclease C Enzyme Solution
(Immediately before use, prepare a solution containing 100 units/ml of Ribonuclease C in Reagent C.)

Enzymatic Assay of RIBONUCLEASE C

PROCEDURE:

Step 1:

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reagent B (Poly C) | 0.996 | 0.996 |

Equilibrate to 25°C. Then add:

| | | |
|-----------------------------|-------|-------|
| Reagent E (Enzyme Solution) | 0.004 | ----- |
| Reagent C (Enz Dil) | ----- | 0.004 |

Immediately mix by swirling and incubate at 25°C for exactly 15 minutes. Then add:

| | | |
|--------------------------------|------|------|
| Reagent D (HClO ₄) | 1.00 | 1.00 |
|--------------------------------|------|------|

Immediately mix by swirling and transfer the solutions to an ice bath and let chill for 2 minutes. Centrifuge for 2 minutes.

Step 2:

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

| | | |
|-------------------|-------|-------|
| Test Supernatant | 0.10 | ----- |
| Blank Supernatant | ----- | 0.10 |
| Deionized Water | 0.90 | 0.90 |

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(2)(1)(\text{df})}{(2)(0.004)(0.1)}$$

2 = Total volume (in milliliters) of stopped reaction (Step 1)

1 = Volume (in milliliters) of Step 2

df = Dilution factor

2 = The increase in $A_{260\text{nm}}$ (arbitrary value) per unit of enzyme as per the Unit Definition

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

0.1 = Volume (in milliliter) of stopped reaction used in

Step 2

Enzymatic Assay of RIBONUCLEASE C

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will produce acid soluble oligonucleotides equivalent to a ΔA_{260} of 2.0 in 15 minutes at pH 7.5 at 25°C, in a reaction volume of 1.0 ml.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 100 mM Tris, 0.075% (w/v) mM polycytidylic acid (5'), 0.05% (w/v) bovine serum albumin, 0.2 mM potassium phosphate, 8 mM potassium chloride, and 0.4 unit ribonuclease C.

REFERENCE:

Schmukler, M., Jewett, P.B., and Levy, C.C. (1975) *Journal of Biological Chemistry* **250**, 2206-2212

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.