

**Enzymatic Assay of RIBONUCLEASE  
from *Aspergillus clavatus***

**PRINCIPLE:**

RNA + H<sub>2</sub>O  $\xrightarrow{\text{Ribonuclease}}$  Acid Soluble Oligonucleotides

Abbreviation used:

RNA = Ribonucleic Acid

**CONDITIONS:** T = 37°C, pH = 7.5, A<sub>260nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- B. 0.2% (w/v) Ribonucleic Acid Solution (RNA)  
(Prepare 5 ml in cold Reagent A using Ribonucleic Acid, Sigma Prod. No. R-6625.)
- C. 25% (v/v) Perchloric Acid Solution (HClO<sub>4</sub>)  
(Prepare 25 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)
- D. 17.7 mM Uranyl Acetate Solution (Uran Acet)  
(Prepare 25 ml in Reagent C using Uranyl Acetate, Dihydrate, Fluka Stock No. 94260.)
- E. Ribonuclease Enzyme Solution  
(Immediately before use, prepare a solution containing 75 - 100 units/ml of Ribonuclease in cold Reagent A.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent B (RNA)	1.40	1.40

Equilibrate to 37°C. Then add:

Reagent E (Enz Soln)	0.08	-----
Reagent A (Buffer)	0.02	0.10

Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

Reagent D (Uran Acet)	0.25	0.25
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Mix by swirling, place in an ice bath for 30 minutes. Centrifuge for 10 minutes.

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.<sup>1</sup>

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{260nm} \text{ Test} - A_{260nm} \text{ Blank}) (5)(1.75)(df)}{(0.08)(1)(0.2)}$$

- 5 = Total volume (in milliliters) of assay
- 1.75 = Volume (in milliliters) of stopped reaction
- df = Dilution factor
- 0.08 = Volume (in milliliter) of enzyme used
- 1 = Extinction coefficient (arbitrary value) as per the Unit Definition
- 0.2 = Volume (in milliliter) of stopped reaction used in the assay

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**CALCULATIONS:** (continued)

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

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

**UNIT DEFINITION:**

One unit will produce acid soluble oligonucleotides equivalent to a  $\Delta A_{260}$  of 1.0 in 30 minutes at pH 7.5 at 37°C in a 1.5 ml reaction volume.

**FINAL ASSAY CONCENTRATIONS:**

In a 1.50 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.2% (w/v) ribonucleic acid, and 6 - 8 units ribonuclease.

**REFERENCE:**

Bauer S., Lamed, R. and Lapidot, Y. (1972) *Biotechnology and Bioengineering* XIV, 861-870

**NOTES:**

1. This reaction is linear between 0 and 0.5 absorbance unit.
2. This assay is based on the cited reference.
3. 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.**