Enzymatic Assay of RIBONUCLEASE U₂
(EC 3.1.27.4)

PRINCIPLE:

\[
\text{Ribonuclease U₂} \quad \text{RNA} + \text{H}_2\text{O} \rightarrow \text{Acid Soluble Oligonucleotides}
\]

Abbreviation used:
RNA = Ribonucleic acid

CONDITIONS:  T = 37°C, pH 4.5, A₂₆₀nm, 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. 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.)

C. 20 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 25 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate.)

D. 25% (v/v) Perchloric Acid Solution (HClO₄)
(Prepare 25 ml in deionized water using Perchloric Acid.)

E. 0.75% (w/v) Uranyl Acetate Solution (Uran Acet)
(Prepare 5 ml in Reagent D using Uranyl Acetate, Dihydrate.)

F. Ribonuclease U₂ Enzyme Solution
(Immediately before use, prepare a solution containing approximately 100 units/ml of Ribonuclease U₂ in cold Reagent A.)
Enzymatic Assay of RIBONUCLEASE U$_2$
(EC 3.1.27.1)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.25</td>
<td>0.40</td>
</tr>
<tr>
<td>Reagent B (RNA)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent C (EDTA)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

Reagent F (Enzyme Solution) 0.15 ------

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

Reagent E (Uran Acet) 0.25 0.25

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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Supernatant</td>
<td>0.20</td>
<td>------</td>
</tr>
<tr>
<td>Blank Supernatant</td>
<td>------</td>
<td>0.20</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>4.80</td>
<td>4.80</td>
</tr>
</tbody>
</table>

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:

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

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 A₂₆₀nm/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 \( \Delta A_{260} \) 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:


NOTES:

1. This assay is based on the cited reference.