Enzymatic Assay of RIBONUCLEASE U₁ (EC 3.1.27.3)

PRINCIPLE:

\[
\text{Ribonuclease U₁} + \text{RNA} + \text{H}_{2}\text{O} \rightarrow \text{Acid Soluble Oligonucleotides}
\]

Abbreviation used:
RNA = Ribonucleic Acid

CONDITIONS:  T = 37°C, pH = 7.5, A_{260nm}, Light path = 1 cm

METHOD:  Spectrophotometric Stop Rate Determination

REAGENTS:

A.  200 mM Tris HCl Buffer, pH 7.5 at 30°C
   (Prepare 100 ml in deionized water using Trizma Hydrochloride. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

B.  1.2% (w/v) Ribonucleic Acid Solution (RNA)
   (Prepare 5 ml in deionized water using Ribonucleic Acid. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

C.  20 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
   (Prepare 5 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate.)

D.  25% (v/v) Perchloric Acid Solution (HClO₄)
   (Prepare 10 ml in deionized water using Perchloric Acid.)

E.  17.7 mM Uranyl Acetate Solution (Uran Acet)
   (Prepare 5 ml in Reagent D using Uranyl Acetate, Dihydrate.)

F.  Ribonuclease U₁ Enzyme Solution
   (Immediately before use, prepare a solution containing 67 - 100 units/ml of Ribonuclease U₁ in cold Reagent A.)
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PROCEDURE:

Step 1:

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

<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>0.25</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 Soln) 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
Reagent F (Enzyme Soln) ------ 0.15

Mix by swirling and incubate to 37°C for 15 minutes. Mix by swirling and centrifuge for 10 minutes.

Step 2:

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

<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 A₂₆₀nm for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(A_{260nm} \text{ Test} - A_{260nm} \text{ Blank})(1.25)(5)(df)}{(1)(0.15)(0.2)}
\]
1.25 = Volume (in milliliters) of stopped reaction (Step 1)
5 = Total volume (in milliliters) of assay (Step 2)
df = Dilution factor
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CALCULATIONS:  (continued)

1 = Extinction coefficient (arbitrary value) as per the Unit Definition
0.15 = Volume (in milliliter) of enzyme used in Step 1
0.2 = Volume (in milliliter) of stopped reaction used in Step 2

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

UNIT DEFINITION:

One unit will produce acid soluble oligonucleotides equivalent to a \(A_{260}\) of 1.0 in 15 minutes at pH 7.5 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 80 mM Tris, 0.30% (w/v) ribonucleic acid, 2 mM ethylenediaminetetraacetic acid, and 10 - 15 units Ribonuclease U₁.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.