Enzymatic Assay of RIBONUCLEASE T<sub>1</sub>  
(EC 3.1.27.3)

**PRINCIPLE:**

Ribonuclease T<sub>1</sub>  
RNA + H<sub>2</sub>O $\rightarrow$ Acid Soluble Oligonucleotides + 3'-GMP

Abbreviations used:  
RNA = Ribonucleic Acid  
3'-GMP = 3'-Guanosine Monophosphate

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

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

A. 200 mM Tris HCl Buffer, pH 7.5 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 37°C with 1 M HCl.)

B. 1.2% (w/v) Ribonucleic Acid Solution (RNA)  
(Prepare 5 ml in Reagent A using Ribonucleic Acid, Sigma Prod. No. R-6625.)

C. 20 mM Ethylenediaminetetraacetic Acid Solution (EDTA)  
(Prepare 25 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

D. 25% (v/v) Perchloric Acid Solution (HClO<sub>4</sub>)  
(Prepare 25 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)

E. 17.6 mM Uranyl Acetate Solution (Uran. Acet.)  
(Prepare 5 ml in Reagent D using Uranyl Acetate, Dihydrate, Fluka Stock No. 94260.)

F. Ribonuclease T<sub>1</sub> Enzyme Solution  
(Immediately before use, prepare a solution containing 70 - 80 units/ml of Ribonuclease T<sub>1</sub> in cold Reagent A.)
Enzymatic Assay of RIBONUCLEASE T₁
(EC 3.1.27.3)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.30</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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Uran. Acet.)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Mix by swirling and allow to stand at room temperature for 15 minutes. Centrifuge for 10 minutes.

Step 2:

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

<table>
<thead>
<tr>
<th>Reagent</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:

\[
\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(1.35)(5)(df)}{(1)(0.1)(0.2)}
\]

1.35 = Volume (in milliliters) of stopped reaction in Step 1
5 = Total volume (in milliliters) of assay in Step 2
df = Dilution factor
1 = Extinction coefficient (arbitrary value) as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used in Step 1
0.2 = Volume (in milliliter) of reaction mix used in Step 2
Enzymatic Assay of RIBONUCLEASE T₁
(EC 3.1.27.3)

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 \( A_{260} \) of 1.0 in 15 minutes at pH 7.5 at 37°C, in a reaction volume of 1 ml. Substrate: Yeast RNA

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 70 mM Tris, 0.30% (w/w) ribonucleic acid, 2 mM ethylenediaminetetraacetic acid, and 7 - 8 units ribonuclease T₁.

REFERENCE:


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.