Enzymatic Assay of RIBONUCLEASE T₂ (EC 3.1.27.1)

PRINCIPLE:

Polyadenylic Acid (5') + H₂O → Acid Soluble Oligonucleotides

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, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 37°C with 1 M HCl.)

B. 1.2% (w/v) Polyadenylic (5') Solution (Poly A)
   (Prepare 5 ml in deionized water using Polyadenylic Acid (5'), Potassium Salt, Sigma Prod. No. P-9403.)

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 (HClO₄)
   (Prepare 25 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)

E. 17.7 mM Uranyl Acetate Solution (Uran Acet)
   (Prepare 5 ml in Reagent D using Uranyl Acetate, Dihydrate, Fluka Stock No. 94260.)

F. Ribonuclease T₂ Enzyme Solution
   (Immediately before use, prepare a solution containing 40 - 80 units/ml in cold Reagent A.)
Enzymatic Assay of RIBONUCLEASE T$_{2}$
(EC 3.1.27.1)

PROCEDURE:

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>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.25</td>
<td>0.35</td>
</tr>
<tr>
<td>Reagent B (Poly A)</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) | 0.10   | ------

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 room temperature for 15 minutes. Centrifuge for 10 minutes.

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

<table>
<thead>
<tr>
<th></th>
<th>Test Supernatant</th>
<th>Blank Supernatant</th>
</tr>
</thead>
<tbody>
<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:

$$\text{Units/ml enzyme} = \frac{(A_{260nm \text{ Test}} - A_{260nm \text{ Blank}})(5)(1.25)(df)}{(0.1)(1)(0.2)}$$

5 = Total volume (in milliliters) of assay
1.25 = Volume (in milliliters) of stopped reaction
df = Dilution factor
0.1 = Volume (in milliliter) of enzyme used
1 = Extinction coefficient (arbitrary value) as per the Unit Definition
0.2 = Volume (in milliliter) of reaction mix used in the assay
Enzymatic Assay of RIBONUCLEASE T<sub>2</sub>  
(EC 3.1.27.1)

**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 \(\text{A}_{260}\) of 1.0 in 15 minutes at pH 4.5 at 37°C in a 1.0 ml reaction volume. Substrate: Polyadenylic Acid (5').

**FINAL ASSAY CONCENTRATIONS:**

In a 1.00 ml reaction mix, the final concentrations are 70 mM sodium acetate, 0.3% (w/v) polyadenylic acid (5'), 2 mM ethylenediaminetetraacetic acid, and 4 - 8 units ribonuclease T<sub>2</sub>.

**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.