Enzymatic Assay of NUCLEASE P₁
(EC 3.1.30.1)
RNA as Substrate

PRINCIPLE:

\[
\text{Nuclease P₁}
\]
\[
\text{RNA + H₂O } \rightarrow \text{Acid Soluble Polynucleotides}
\]

Abbreviation used:
RNA = Ribonucleic Acid

CONDITIONS:  \( T = 37{}^\circ \text{C}, \text{pH} = 5.3, A_{260nm}, \text{Light path} = 1 \text{ cm} \)

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 0.59\% (w/v) Barbital Acetate Buffer, pH 5.3 at 37\°C
(Prepare 25 ml in deionized water using Barbital Buffer, Sigma Prod. No. B-6632. Adjust to pH 5.3 at 37\°C with 1 M HOAc.)

B. 0.2\% (w/v) Ribonucleic Acid Solution (RNA)
(Prepare 4 ml in deionized water using Ribonucleic Acid, Sigma Prod. No. R-6625.)

C. 2.5\% (v/v) Perchloric Acid Solution (HClO₄)
(Prepare 5 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)

D. 0.25\% (w/v) Uranyl Acetate Solution (Uran Acet)
(Prepare 5 ml in Reagent C using Uranyl Acetate, Fluka Prod. No. 94260. Store on ice.)

E. Nuclease P₁ Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Nuclease P₁ in cold deionized water.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent B (RNA)</td>
<td>0.20</td>
<td>0.20</td>
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</tbody>
</table>

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

| Reagent E (Enzyme Solution) | 0.10 | ------ |
| Deionized Water             | ------ | 0.10 |

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

| Reagent D (Uran Acet) | 0.50 | 0.50 |

Mix by swirling and place in an ice bath for 20 minutes. Centrifuge for 5 minutes. Remove 0.80 ml of the supernatant and dilute to 2.80 ml with deionized water. Transfer the solutions to suitable cuvettes and obtain the \( A_{260nm} \) for the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(A_{260nm} \text{ Test} - A_{260nm} \text{ Blank})(1)(2.8)(df)}{(10.6)(15)(0.1)(0.8)}
\]

1 = Volume (in milliliter) of stopped reaction
2.8 = Final volume (in milliliters) of assay
df = Dilution factor
10.6 = Millimolar extinction coefficient of hydrolyzed ribonucleic acid at 260 nm
15 = Time (in minutes) of assay as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used
0.8 = Volume (in milliliter) of stopped reaction used in the spectrophotometric determination

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

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

One unit will liberate 1.0 µmole of acid soluble nucleotides from RNA (R6625) per minute at pH 5.3 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.50 ml reaction mix, the final concentrations are 0.24% (w/v) barbital, 0.08% (w/v) ribonucleic acid, and 0.01 - 0.02 unit nuclease P₁.

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.