Enzymatic Assay of ENDONUCLEASE

PRINCIPLE:

\[ \text{DNA} + \text{H}_2\text{O} \xrightarrow{\text{Endonuclease}} \text{Acid-Soluble Oligonucleotides} \]

Abbreviations:
DNA = Deoxyribonucleic Acid

CONDITIONS: \( T = 37^\circ\text{C}, \ \text{pH} = 8.0, A_{260nm}, \ \text{Light path} = 1 \text{ cm} \)

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Tris HCl Buffer with 1 mM Magnesium Chloride and 0.1% (w/v) Bovine Serum Albumin, pH 8.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503, Magnesium Chloride, Hexahydrate, Prod. No. M-0250, and Albumin, Bovine, Prod. No. A-4503 or equivalent. Adjust to pH 8.0 at 37°C with 1 M HCl.)

B. 0.1% (w/v) Deoxyribonucleic Acid (DNA)
(Prepare 25 ml in Reagent A, using Deoxyribonucleic Acid, Sodium Salt, Prod. No. D-1626.)

C. 4% (v/v) Perchloric Acid Solution (HClO₄)
(Prepare 10 ml in deionized water using Perchloric Acid, Stock No. 24425-2.)

D. Endonuclease Enzyme Solution
Immediately before use, prepare a solution containing 40 units/ml of Endonuclease in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent B (DNA)</td>
<td>2.50</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:
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PROCEDURE: (continued)

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.125</td>
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</tr>
<tr>
<td>Reagent A (Buffer)</td>
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<td>0.125</td>
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Mix by inversion and incubate for exactly 30 minutes at 37°C. Remove 0.50 ml from both the Test and Blank and transfer into Eppendorf tubes containing 0.50 ml of ice cold Reagent C (HClO₄). Cool on ice for 60 minutes.

Centrifuge for 5 minutes at 4°C. Obtain the A₂₆₀nm of the supernatant for both the Test and Blank in a suitable spectrophotometer using air as the reference.

CALCULATION:

\[
\text{Units/ml enzyme} = \frac{(A₂₆₀nm \text{ Test} - A₂₆₀nm \text{ Blank})(2.625)(df)}{(1)(0.5)(0.125)}
\]

2.625 = Total volume (in milliliters) of assay
df = Dilution factor
1 = A₂₆₀nm of one unit as per the Unit Definition
0.5 = Volume (in milliliter) of supernatant used in assay
0.125 = Volume (in milliliter) of enzyme used

UNIT DEFINITION:

One unit will produce acid-soluble oligonucleotides equivalent to a ΔA₂₆₀nm of 1.0 in 30 minutes at pH 8.0 at 37°C (reaction volume 2.625 ml).

FINAL ASSAY CONCENTRATION:

In a 2.625 ml reaction mix, the final concentrations are 50 mM Tris, 1 mM magnesium chloride, 0.1% (w/v) bovine serum albumin, 0.095% (w/v) deoxyribonucleic acid and 5 units endonuclease.

NOTES:

1. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.
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This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.