

Enzymatic Assay of ENDONUCLEASE

PRINCIPLE:

DNA + H₂O ^{Endonuclease} > Acid-Soluble Oligonucleotides

Abbreviations:

DNA = Deoxyribonucleic Acid

CONDITIONS: T = 37°C, pH = 8.0, A_{260nm}, Light path = 1 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:

	<u>Test</u>	<u>Blank</u>
Reagent B (DNA)	2.50	2.50

Equilibrate to 37°C. Then add:

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PROCEDURE: (continued)

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.125	-----
Reagent A (Buffer)	-----	0.125

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 A_{260nm} of the supernatant for both the Test and Blank in a suitable spectrophotometer using air as the reference.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(2.625)(\text{df})}{(1)(0.5)(0.125)}$$

2.625 = Total volume (in milliliters) of assay

df = Dilution factor

1 = A_{260nm} 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 ΔA_{260nm} 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.