

**Enzymatic Assay of ENDONUCLEASE
from Neurospora crassa**

PRINCIPLE:

DNA + H₂O Endonuclease > Acid-Soluble Oligonucleotides

Abbreviation:

DNA = Deoxyribonucleic Acid

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

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 200 mM Tris HCl Buffer with 20 mM Magnesium Chloride and 300 mM Sodium Chloride, pH 8.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 8.0 at 37°C with 1 M HCl.)
- B. 0.08% (w/v) Deoxyribonucleic Acid (DNA)
(Prepare by dissolving 15 mg of Deoxyribonucleic Acid, Sodium Salt, Sigma Prod. No. D-1501, in 5 ml of deionized water. Mix by stirring for 4 - 5 hours at room temperature, then heat in a boiling water bath for 15 minutes. Cool to room temperature and then remove 2.7 ml of the DNA solution and combine with 5 ml of Reagent A and 2.3 ml of deionized water.)
- C. 1 M Perchloric Acid Solution (HClO₄)
(Prepare 10 ml in deionized water using Perchloric Acid, Sigma Stock No. 24,425-2.)
- D. Endonuclease Enzyme Solution
(Immediately before use, prepare a solution containing 16 - 48 units/ml of Endonuclease in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (DNA)	1.00	1.00
Deionized Water	0.50	0.50

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

Reagent D (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Mix by inversion and incubate for exactly 30 minutes at 37°C. Remove 0.30 ml from both the Test and Blank and transfer into Eppendorf tubes each containing 0.50 ml of Reagent C (HClO₄) and 0.20 ml of deionized water.

Mix by swirling and centrifuge for 10 minutes. Remove 0.10 ml of the supernatant from both the Test and Blank and add 2.90 ml of deionized water to each tube. Record the A_{260nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(A_{260\text{nm}} \text{ Test} - A_{260\text{nm}} \text{ Blank})(3)(1.60)(\text{df})}{(0.1)(0.3)(0.1)}$$

- 3 = Total volume (in milliliters) used to measure the A_{260nm}
- 1.60 = Volume (in milliliters) of reaction mix
- 0.1 = Volume (in milliliter) of enzyme used
- 0.3 = Volume (in milliliter) removed from the reaction mix
- 0.1 = Volume (in milliliter) removed from the stopped reaction

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce acid-soluble oligonucleotides equivalent to a ΔA_{260nm} of 1.0 in 30 minutes at pH 8.0 at 37°C in a reaction volume of 1.60 ml.

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FINAL ASSAY CONCENTRATIONS:

In a 1.60 ml reaction mix, the final concentrations are 63 mM Tris, 6 mM magnesium chloride, 94 mM sodium chloride, 0.05% (w/v) deoxyribonucleic acid, and 1.6 - 4.8 units endonuclease.

REFERENCE:

Fraser, M.J. (1980) *Methods in Enzymology* **65**, Part 1, 255-263

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.