

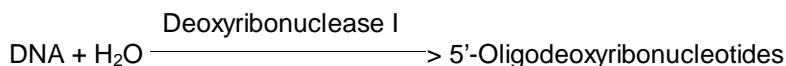


SIGMA QUALITY CONTROL TEST

Product Information

Enzymatic Assay of DEOXYRIBONUCLEASE I (EC 3.1.21.1)

PRINCIPLE:



Abbreviation used:

DNA = Deoxyribonucleic Acid

CONDITIONS: T = 25°C, pH = 5.0, $A_{260\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1 M Sodium Acetate Buffer, pH 5.0 at 25°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 25°C with 5 M HCl.)
- B. 100 mM Magnesium Sulfate Solution (MgSO_4)
(Prepare 5 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- C. 0.033% (w/v) Deoxyribonucleic Acid Solution (DNA)
(Dissolve a 1 mg vial of Deoxyribonucleic Acid, Sodium, Sigma Prod. No. D-3664, in 3.1 ml of deionized water.)
- D. 150 mM Sodium Chloride (NaCl)
(Prepare 50 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- E. Deoxyribonuclease I Enzyme Solution (DNase I)
(Immediately before use, prepare a solution containing 400 - 500 Kunitz units/ml in cold Reagent D.)
- F. Standard DNase Solution (Std Soln)¹
(Reconstitute a vial of Deoxyribonuclease I, Standardized vial, containing 2000 Kunitz units, Sigma Prod. No. D-4263, with 1 ml of cold Reagent D. Immediately before use, dilute to 400-500 Kunitz units/ml with cold Reagent D.)

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PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagent into a suitable container:

Reagent A (Buffer)	2.50
Reagent B (MgSO ₄)	1.25
Reagent C (DNA)	3.00
Deionized Water	18.25

Mix by gentle stirring and adjust to pH 5.0 at 25°C with 1 M HCl or 1 M NaOH, if necessary. Then pipette (in milliliters) the following reagents into suitable quartz cuvettes:

	<u>Test</u>	<u>Std</u>	<u>Blank</u>
Reagent D (NaCl)	-----	-----	0.50
Reaction Cocktail	2.50	2.50	2.50

Mix by inversion and equilibrate to 25°C. Monitor the A_{260nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (DNase I)	0.50	-----	-----
Reagent F (Std Soln)	-----	0.50	-----

Immediately mix by inversion and record the increase in A_{260nm} for approximately 10 minutes. Obtain the ΔA_{260nm}/minute using the maximum linear rate for the Test, Standard, and Blank.

CALCULATIONS:

$$\text{Units/ml Test Soln} = \frac{(\Delta A_{260\text{nm}}/\text{min Test} - \Delta A_{260\text{nm}}/\text{min Blank}) (3) (df)}{(0.001) (0.5)}$$

$$\text{Units/ml Std Soln} = \frac{(\Delta A_{260\text{nm}}/\text{min Std} - \Delta A_{260\text{nm}}/\text{min Blank}) (3) (df)}{(0.001) (0.5)}$$

- 3 = Volume (in milliliters) of assay
- df = Dilution factor (includes dilution of the standard vial)
- 0.001 = ΔA_{260nm} as per the Unit Definition
- 0.5 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

$$CF = \frac{(2000)}{\text{Experimental units/Std vial}}$$

CF = Correction Factor
2000 = Theoretical units/Std vial

Corrected Units/ml enzyme = (CF) (Units/ml Test Solution)

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

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

UNIT DEFINITION:

One Kunitz unit will produce a ΔA_{260} of 0.001 per minute per ml at pH 5.0 at 25°C, using DNA, Types I or III as substrate. $[Mg^{++}] = 4.2 \text{ mM}$.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM sodium acetate, 4.2 mM magnesium sulfate, 25 mM sodium chloride, 0.003% (w/v) deoxyribonucleic acid, and 200 - 250 units deoxyribonuclease I.

REFERENCE:

- Kunitz, M. (1950) *Journal of General Physiology* **33**, 349-362
- Kunitz, M. (1950) *Journal of General Physiology* **33**, 363-377
- Lindberg, U. (1964) *Biochimica et Biophysica Acta* **82**, 237-248

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NOTES:

1. There is no absolute standard for the assay of DNase. When the procedure of Kunitz is used, the result is affected by the particular lot of substrate used. For DNase studies, we offer a standard DNase I vial, Sigma Prod. No. D-4263, which has been standardized to contain 2,000 Kunitz units using our DNA, Sigma Prod. No. D-1501 as substrate.
2. This assay is based on the cited references.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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