SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of DEOXYRIBONUCLEASE II
(EC 3.1.22.1)

PRINCIPLE:

$$DNA + H_2O \xrightarrow{\text{Deoxyribonuclease II}} > 3'\text{-Oligodeoxyribonucleotides}$$

Abbreviation used:
DNA = Deoxyribonucleic Acid

CONDITIONS: T = 25°C, pH 4.6, A_{260nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 1 M Sodium Acetate Buffer
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.6 at 25°C with 6 N HCl.)

B. 20 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 25 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

C. 0.033% (w/v) Deoxyribonucleic Acid Solution (DNA)
(Prepare 30 ml in deionized water using Deoxyribonucleic Acid, Sodium Salt, Sigma Prod. No. D-1501. Gently mix by stirring at 4°C for 15 - 24 hours.)

D. 150 mM Sodium Chloride Solution (NaCl)
(Prepare 25 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)

E. Standard DNase Solution (Std Soln)[1]
(Immediately before use, reconstitute a vial of Deoxyribonuclease II, Standardized vial, containing approximately 1500 Kunitz units, Sigma Prod. No. D-9784, with 2 ml of cold deionized water.)

F. Deoxyribonuclease II Enzyme Solution (DNase II)
(Immediately before use, prepare a solution containing 500 - 1000 Kunitz units/ml in cold deionized water.)

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PROCEDURE:

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

Reagent A (Buffer)	7.50
Reagent B ($MgSO_4$)	3.75
Reagent C (DNA)	9.00
Deionized water	54.75

Mix by swirling and equilibrate to 25°C.

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

	Test	Std	Blank
Reaction Cocktail	2.50	2.50	2.50
Reagent D (NaCl)	0.40	0.40	0.40

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

Reagent E (Std Soln)	----	0.10	----
Reagent F (DNase II)	0.10	----	----
Deionized Water	----	----	0.10

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:

$$\text{Units/ml Test Solution} = \frac{(\Delta A_{260nm}/\text{min Test} - \Delta A_{260nm}/\text{min Blank})(3)(df)}{(0.001)(0.1)}$$

$$\text{Units/ml Std Solution} = \frac{(\Delta A_{260nm}/\text{min Std} - \Delta A_{260nm}/\text{min Blank})(3)(df)}{(0.001)(0.1)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
0.001 = ΔA_{260nm} per unit as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used

CALCULATIONS: (continued)

CF = Correction factor

$$CF = \frac{750}{\text{Experimental Units/ml Std Soln}}$$

750 = Theoretical Units/ml Std Soln

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 4.6 at 25°C.
$[Mg^{++}]$ = 0.83 mM

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM sodium acetate, 0.83 mM magnesium sulfate, 0.0033% (w/v) deoxyribonucleic acid, 20 mM sodium chloride, and 50 - 100 units deoxyribonuclease II.

REFERENCES:

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

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 II vial, Sigma Prod. No. D-9784, which has been standardized to contain approximately 1500 Kunitz units (actual contents listed on the label) using Deoxyribonucleic Acid, Sodium Salt, Sigma Prod. No. D-1501 as substrate.

NOTES:

2. This assay is based on the cited references.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.