SIGMA

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of NUCLEASE, MICROCOCCAL (EC 3.1.31.1)

PRINCIPLE:

DNA + H_2O $\xrightarrow{\text{Nuclease, Micrococcal}}$ Acid Soluble Polynucleotides

CONDITIONS: T = 37°C, pH = 8.8, A_{260nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Borate Buffer, pH 8.8 at 37°C
(Prepare 25 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 8.8 at 37°C with 1 M NaOH.)

B. 10 mM Sodium Chloride Solution (NaCl)
(Prepare 50 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)

C. 0.25% (w/v) Deoxyribonucleic Acid Solution (DNA)
(Prepare 5 ml in Reagent B using Deoxyribonucleic Acid, Sodium Salt, Sigma Prod. No. D-1501. Gentle agitation may be required to obtain complete dissolution[1].)

D. 10 mM Calcium Chloride Solution ($CaCl_2$)
(Prepare 10 ml in deionized water using Calcium Chloride Dihydrate, Sigma Prod. No. C-3881.)

E. 0.1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 100 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-7888.)

F. Nuclease, Micrococcal Enzyme Solution[2]
(Immediately before use, prepare a solution containing 0.04 - 0.08 unit/ml of Micrococcal Nuclease in cold Reagent E.)

G. 7% (v/v) Perchloric Acid Solution ($HClO_4$)
(Prepare 10 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.20	0.20
Reagent C (DNA)	0.20	0.20
Reagent D ($CaCl_2$)	0.10	0.10

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

Reagent E (BSA)	------	0.20
Reagent F (Enzyme)	0.20	------

Mix by swirling and incubate for exactly 30 minutes at 37°C. Then add:

Reagent G ($Hcl0_4$)	1.00	1.00

Place tubes in an ice bath for 10 minutes. Then add:

Deionized water	5.40	5.40

Mix by swirling and centrifuge for 10 minutes. Transfer the supernatants from both the Test and Blank to suitable cuvettes and record the A_{260nm} for each using a suitable spectrophotometer.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(A_{260nm}\ \text{Test} - A_{260nm}\ \text{Blank})(7.1)(df)}{(10)(30)(0.2)}$$

7.1 = Total volume (in milliliters) of assay
df = Dilution factor
10 = Millimolar extinction coefficient of soluble polynucleotides at 260 nm.
30 = Time (in minutes) of the assay as per the Unit Definition
0.2 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will produce 1 µmole of acid soluble polynucleotides from native DNA per minute at pH 8.8 at 37°C, based on E^{M}_{260} = 10,000 for the mixed nucleotides.

FINAL ASSAY CONCENTRATION:

In a 0.70 ml reaction mix, the final concentrations are 29 mM borate, 2.9 mM sodium chloride, 0.07% (w/v) deoxyribonucleic acid, 1.4 mM calcium chloride, 0.03% (w/v) bovine serum albumin and .008 - .016 unit of nuclease, micrococcal.

REFERENCE:

Alexander, M., Heppel L.A. and Hurwitz J. (1961) *Journal of Biological Chemistry* **236**, 3014-3019

NOTE:

1. Stir at 4°C until complete dissolution is obtained. This may take several hours. Do not store overnight to dissolve the DNA.

2. The enzyme concentration must be kept at approximately 1 mg protein per ml until just prior to dilution for use in the reaction mix.

3. This assay is based on the cited reference.

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