

Enzymatic Assay of CUCUMISIN

PRINCIPLE:

Casein + H₂O $\xrightarrow{\text{Protease}}$ Amino Acids

CONDITIONS: T = 37°C, pH = 10.0, A_{275nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Sodium Tetraborate Solution
(Prepare 10 ml in deionized water using Borax, Sigma Prod. No. B-9876.)
- B. 0.6% (w/v) Casein with 10.0 mM Sodium Tetraborate, pH 11.0 at 37°C (Casein).
(Prepare by dissolving 600 mg of Casein, Sigma Prod. No. C-7078 in 4 ml of 0.1 M NaOH in a hot water bath (60°C). Cool to room temperature and then add 10 ml of Reagent A and 80 ml of deionized water. Adjust to pH 10.0 at 37°C with 1 M NaOH and bring to a volume of 100 ml with deionized water.)
- C. 110 mM Trichloroacetic Acid with 220 mM Sodium Acetate and 330 mM Acetic Acid (TCA)
(Prepare 40 ml in deionized water using Trichloroacetic Acid, 6.1 N, approximately 100% (w/v), Sigma Stock No. 490-10, Sodium Acetate Trihydrate, Sigma Prod. No. S-8625, and Acetic Acid, Glacial, Sigma Prod. No. A-6283.)
- D. 10.0 mM Sodium Tetraborate Buffer, pH 10.0 at 37°C (Enz Dil I)
(Prepare 10 ml in deionized water using Borax, Sigma Prod. No. B-9876. Adjust to pH 10.0 at 37°C with 1 M NaOH. Adjust to pH 10.0 at 37°C with 1 M NaOH.)
- E. 2.0 mM Calcium Acetate Solution (Enz Dil II)
(Prepare 10 ml in deionized water using Calcium Acetate, Sigma Prod. No. C-1000.)

Enzymatic Assay of CUCUMISIN

REAGENTS:

- F. Cucumisin Enzyme Solution
(Prepare by dissolving 1 mg of Cucumisin in 2 ml of ice-cold Reagent D. Immediately before use, dilute to 0.20 - 0.40 unit/ml of Cucumisin with Reagent E.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Casein)	3.00	3.00

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.50	-----
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Immediately mix by swirling and incubate at 37°C for exactly 10 minutes. Then add:

Reagent C (TCA)	3.20	3.20
Reagent F (Enzyme Solution)	-----	0.50

Mix by swirling and incubate at 37°C for 20 minutes. Filter the solutions through Whatman #50 filter paper and transfer the solutions to suitable cuvettes and record the $A_{275\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{275\text{nm}} \text{ Test} - A_{275\text{nm}} \text{ Blank})(6.7)(\text{df})}{(10)(1.34)(0.5)}$$

6.7 = Total volume (in milliliters) of assay

df = Dilution factor

10 = Time (in minutes) of the assay per the Unit Definition

1.34 = Millimolar extinction coefficient¹ of tyrosine at 275nm under the conditions of this assay

0.5 = Volume (in milliliter) of enzyme used

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

Enzymatic Assay of CUCUMISIN

CALCULATIONS:

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

UNIT DEFINITION:

One unit will hydrolyze casein to produce peptides equivalent to 1.0 μ mole of tyrosine per minute at pH 10.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.50 ml reaction mix, the final concentrations are 0.5% (w/v) casein, 9 mM sodium tetraborate, 0.3 mM calcium acetate, and 0.10 - 0.2 unit cucumisin.

REFERENCE:

Nakanishi, T., Matsumura, Y., Minamiura, N., and Yamamoto, T. (1974) *Agricultural Biological Chemistry* **38**, 37-44

Kaneda, M. and Tominaga, N. (1975) *Journal of Biochemistry* **78**, 1287-1296

NOTES:

1. This value was experimentally determined by Sigma.
2. This assay is based on the cited references.
3. 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.