## Enzymatic Assay of METALLOENDOPEPTIDASE

**PRINCIPLE:**

Casein + $H_2O$ $\xrightarrow{\text{Metalloendopeptidase}}$ Amino Acids

**CONDITIONS:** T = 30°C, pH 11.0, $A_{275nm}$, Light path = 1 cm

**METHOD:** Spectrophotometric Stopped 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 30°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 11.0 at 30°C with 1 M NaOH and bring to a volume of 100 ml with deionized water.)

C. 110 mM Trichloroacetic Acid Solution 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. 50 mM Potassium Phosphate Buffer, pH 7.0 at 30°C (Enz Dil)
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 30°C with 1 M KOH.)

E. Metalloendopeptidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.25 unit/ml of Metalloendopeptidase in cold Reagent D.)

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**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent B (Casein) | 3.00 | 3.00 |

Equilibrate to 30°C. Then add:

| | | |
|---|---|---|
| Reagent E (Enzyme Solution) | 0.10 | ------ |

Immediately mix by swirling and incubate at 30°C for exactly 3 minutes.[1] Then add:

| | | |
|---|---|---|
| Reagent C (TCA) | 3.20 | 3.20 |
| Reagent E (Enzyme Solution) | ------ | 0.10 |

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

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{275nm}\ \text{Test} - A_{275nm}\ \text{Blank})(6.3)(df)}{(3)(1.34)(0.1)}$$

6.3 = Total volume (in milliliters) of the stopped reaction
df = Dilution factor
3 = Time (in minutes) of assay per the Unit Definition
1.34 = Millimolar extinction coefficient of tyrosine under the conditions of this assay
0.1 = Volume (in milliliter) of enzyme used

**UNIT DEFINITION:**

One unit will hydrolyze casein to produce peptide equivalent to 1.0 µmole of tyrosine per minute at pH 11.0 at 30°C.

**FINAL ASSAY CONCENTRATION:**

In a 3.10 ml reaction mix, the final concentrations are 0.58% (w/v) casein, 9.7 mM sodium tetraborate,

1.6 mM potassium phosphate, and 0.025 unit metalloendopeptidase.

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**REFERENCE:**

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

**NOTES:**

1. The assay is also run for 2 minutes. The assay time which gives the most linear response is used in the calculation.

2. This assay is based on the cited reference.

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