

## Enzymatic Assay of CATHEPSIN C (EC 3.4.14.1)

### PRINCIPLE:

GPAA + Hydroxylamine  $\xrightarrow{\text{Cathepsin C}}$  Glycyl-phenylalanylhydroxamic acid + NH<sub>3</sub>

Abbreviations:

GPAA = Glycyl-L-phenylalaninamide

**CONDITIONS:** T = 37°C, pH 6.8, A = 510 nm, Light path = 1 cm

**METHOD:** Colorimetric

### REAGENTS:

- A. 2000 mM Hydroxylamine HCl Buffer, pH 6.8 at 37°C  
(Prepare 100 ml by first making a 4000 mM stock solution of Hydroxylamine HCl in deionized water using Hydroxylamine Hydrochloride, Prod. No. H-9876. Adjust to pH 6.8 at 37°C with concentrated NaOH and dilute to 2000 mM just prior to use. **PREPARE FRESH.**)
- B. 250 mM Glycyl-L-Phenylalaninamide Acetate Solution, pH 6.8 at 37°C (GPAA)  
(Prepare 10 ml in deionized water using GLY-PHE Amide, Acetate Salt, Prod. No. G-2877. Adjust to pH 6.8 at 37°C with 0.1 M NaOH. **PREPARE FRESH.**)
- C. 125 mM β-Mercaptoethylamine Solution, pH 6.8 at 37°C (2-MEA)  
(Prepare 10 ml in deionized water using 2-Mercaptoethylamine Hydrochloride, Prod. No. M-6500. Adjust to pH 6.8 at 37°C with 0.1 M NaOH. **PREPARE FRESH.**)
- D. 20% (v/v) Trichloroacetic Acid Solution (TCA)  
(Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, Stock No. 490-10.)
- E. 5% (w/v) Ferric Chloride Solution (FeCl<sub>3</sub> · 6 H<sub>2</sub>O)  
(Prepare 25 ml in 0.1 M HCl using Ferric Chloride, Hexahydrate, Prod. No. F-2877.)

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**REAGENTS:** (continued)

- F. Cathepsin C Enzyme Solution  
(Immediately before use, prepare a solution containing 1.5 - 3.0 units/ml of Cathepsin C in cold deionized water.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.10	0.10
Reagent B (GPAA)	0.10	0.10
Reagent C (2-MEA)                      0.10	0.10	
Deionized Water	0.10	0.10

Mix by inversion and equilibrate to 37°C, for no more than 5 minutes. Then add:

Reagent F (Enzyme Solution)	0.10	-----
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Mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

Reagent D (TCA)	0.50	0.50
Reagent E (FeCl <sub>3</sub> A 6 H <sub>2</sub> O)	0.50	0.50
Reagent F (Enzyme)	-----	0.10
Deionized Water	1.50	1.50

Mix by inversion. If turbidity occurs upon the addition of the TCA, samples should be centrifuged. Transfer the solution to suitable cuvettes and record the A<sub>510nm</sub> within 10 minutes for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{510\text{nm}} \text{ Test} - A_{510\text{nm}} \text{ Blank})(3.0)(\text{df})}{(0.37)(10)(0.1)}$$

3.0 = Volume of assay (ml)

df = Dilution factor

0.37 = Millimolar extinction coefficient of phenylalanine hydroxamate at 510 nm.

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

0.1 = 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 1.0  $\mu$ mole of hydroxamic acid from glycy-L-phenylalaninamide and hydroxylamine per minute at pH 6.8 at 37°C using DL-phenylalanine hydroxamate as the standard. In addition to its hydrolytic properties cathepsin C catalyzes the polymerization of dipeptide amides.

**FINAL ASSAY CONCENTRATION:**

In a 0.50 ml reaction mix, the final concentrations are 400 mM hydroxylamine, 50 mM glycy-L-phenylalaninamide acetate, 25 mM 2-mercaptoethylamine and 0.15 - 0.30 unit cathepsin C.

**REFERENCE:**

Metrione, R.M. Neves, A.G., and Fruton, J.S. (1966) *Biochemistry* **5**, 1597.

**NOTES:**

1. This assay is based on the cited reference.
2. 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.**