

## Enzymatic Assay of CATHEPSIN D (EC 3.4.23.5)

### PRINCIPLE:

Acid Denatured Hemoglobin  $\xrightarrow{\text{Cathepsin D}}$  TCA-Soluble Peptides

**CONDITIONS:** T = 37°C, pH = 3.3, A<sub>280nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

### REAGENTS:

- A. 100 mM Formic Acid Buffer, pH 3.3 at 37°C  
(Prepare 100 ml in deionized water using Formic Acid, Sodium Salt, Sigma Prod. No. F-6502.  
Adjust to pH 3.3 at 37°C with 1 M HCl or 1 M NaOH.)
- B. 2.5% (w/v) Hemoglobin Solution (Hb)  
(Prepare 10 ml in Reagent A using Hemoglobin, Sigma Prod. No. H-2625.)
- C. 10% (v/v) Trichloroacetic Acid Solution (TCA)  
(Prepare 100 ml in deionized water using Trichloroacetic Acid, 6.1 N solution, approximately  
100% (2/v) Sigma Stock No. 490-10.)
- D. Cathepsin D Enzyme Solution  
(Immediately before use, prepare a solution containing 5-15 units/ml if Cathepsin D in cold  
Reagent A.)

### PROCEDURE:

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

|                    | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reagent A (Buffer) | 0.73        | 0.73         |
| Reagent B (Hb)     | 0.05        | 0.05         |

Equilibrate to 37°C. Then add:

|                             |      |       |
|-----------------------------|------|-------|
| Reagent D (Enzyme Solution) | 0.02 | ----- |
|-----------------------------|------|-------|

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

|                             |       |      |
|-----------------------------|-------|------|
| Reagent (TCA)               | 0.50  | 0.50 |
| Reagent D (Enzyme Solution) | ----- | 0.02 |

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### PROCEDURE: (continued)

Mix by inversion and incubate for 10 minutes at 37°C. Filter the solutions through a 0.2 µM syringe filter. Transfer the solution to suitable cuvettes and record the  $A_{280\text{nm}}$  for both the Test and Blank using a suitable spectrophotometric.

### CALCULATIONS:

$$\text{Units/vial enzyme} = \frac{(A_{280\text{nm}} \text{ Test} - A_{280\text{nm}} \text{ Blank})(1.3)(\text{df})}{(1)(0.02)}$$

1.3 = Total volume (in milliliters) of assay

df = Dilution factor of vial

1 = Increase in  $A_{280\text{nm}}$  for 30 minutes as per the Unit Definition

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

### FINAL ASSAY CONCENTRATION:

In a 0.8 ml reaction mix, the final concentrations are 100 mM formic acid, 0.16% (w/v) hemoglobin and 0.1-0.3 units cathepsin B.

### UNIT DEFINITION:

One unit will produce an increase in  $A_{280\text{nm}}$  of 1.0 in 30 minutes at pH 3.3 at 37°C measured as TCA-soluble products using acid denatured hemoglobin as substrate (1 cm light path).

### REFERENCE:

Barrett, A.J. (1970) *Biochemical Journal* 117, 601-607

### 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.**