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, $A_{280\text{nm}}$, 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.
   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.)

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) .)

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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>Reagent B (Hb)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Regent D (Enzyme Solution)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (TCA)</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Enzymatic Assay of CATHEPSIN D  
(EC 3.4.23.5)

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 $A_{280nm}$ for both the Test and Blank using a suitable spectrophotometric.

CALCULATIONS:

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

1.3 = Total volume (in milliliters) of assay
df = Dilution factor of vial
1 = Increase in $A_{280nm}$ 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 $A_{300nm}$ 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:


NOTES:

1. This assay is based on the cited reference.
2. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.