Enzymatic Assay of CATHEPSIN C
(EC 3.4.14.1)

PRINCIPLE:

GPAA + Hydroxylamine → Glycyl-phenylalanylhydroxamic acid + NH₃

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.  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 37EC (GPAA)
(Prepare 10 ml in deionized water using GLY-PHE Amide, Acetate Salt.  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 37EC (2-MEA)
(Prepare 10 ml in deionized water using 2-Mercaptoethylamine Hydrochloride.  Adjust to pH 6.8 at 37°C with 0.1 M NaOH. ***PREPARE FRESH.***)

D.  20% (v/v) Trichloracetic Acid Solution (TCA)
(Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution.)

E.  5% (w/v) Ferric Chloride Solution (FeCl₃ · 6 H₂O)
(Prepare 25 ml in 0.1 M HCl using Ferric Chloride, Hexahydrate.)
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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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (GPAA)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (2-MEA)</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

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

Mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (TCA)</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent E (FeCl₃ 6 H₂O)</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent F (Enzyme)</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.50</td>
</tr>
</tbody>
</table>

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 A₅₁₀nm within 10 minutes for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

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

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)

\[
\text{Units/mg solid} = \frac{\text{mg solid/ml enzyme} \times \text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]

\[
\text{Units/mg protein} = \frac{\text{mg protein/ml enzyme} \times \text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

UNIT DEFINITION:

One unit will produce 1.0 µmole of hydroxamic acid from glycyl-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 glycyl-L-phenylalaninamide acetate, 25 mM 2-mercaptoethylamine and 0.15 - 0.30 unit cathepsin C.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.