SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of CATHEPSIN B
(EC 3.4.22.1)
Sigma Prod. No. C-6286

PRINCIPLE:

\[
\text{Na-CBZ-L-Lysine p-Nitrophenyl Ester} + \text{H}_2\text{O} \xrightarrow{\text{Cathepsin B}} \text{Na-CBZ-L-Lysine} + \text{p-Nitrophenol}
\]

Abbreviation used:
CBZ = N-Carbobenzoxy

CONDITIONS: \( T = 25^\circ\text{C}, \ pH = 5.0, \ A_{326\text{nm}}, \ \text{Light path} = 1 \ \text{cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 20 mM Sodium Acetate Buffer with 1.0 mM Ethylenediaminetetraacetic Acid and 5.0 mM L-Cysteine
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.0 at 25°C with 1 M NaOH.)

B. Dimethyl Sulfoxide Solution (DMSO)
(Use Dimethyl Sulfoxide, Sigma Prod. No. D-5879.)

C. 5.2 mM Na-CBZ-L-Lysine p-Nitrophenyl Ester Solution (Substrate)

D. Cathepsin B Enzyme Solution
(Immediately before use, prepare a solution containing 2.5 - 5.0 units/ml of Cathepsin B in Reagent A.)
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PROCEDURE:  

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent C (Substrate)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the rate of increase in the absorbance at 326 nm for at least two minutes but no more than three minutes using a suitably thermostatted spectrophotometer. This rate should be approximately 0.03 absorbance units per minute. Then add:  

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.01</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A$_{326\text{nm}}$ for approximately 3 minutes. Obtain the $\Delta A_{326\text{nm}}$/minute using the maximum linear rate for both the Test and the Blank.  

CALCULATIONS:  

\[
\text{Units/ml enzyme} = (\Delta A_{326\text{nm}}/\text{min Test} - \Delta A_{326\text{nm}}/\text{min Blank})(3.06)(df)
\]

\[
3.06 = \text{Total volume (in milliliters) of assay}
\]

\[
df = \text{Dilution factor}
\]

\[
7.58 = \text{Millimolar extinction coefficient of p-nitrophenol at 326 nm}
\]

\[
0.01 = \text{Volume (in milliliter) of enzyme used}
\]

\[
\text{units/ml enzyme}
\]

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

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

UNIT DEFINITION:  

One unit will hydrolyze 1 ìmole of Ná-CBZ-lysine p-nitrophenyl ester per minute at pH 5.0 at 25°C.
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FINAL ASSAY CONCENTRATION:  

In a 3.06 ml reaction mix, the final concentrations are 20 mM sodium acetate, 0.98 mM ethylenediaminetetraacetic acid, 4.9 mM L-cysteine, 0.08 mM Nα-CBZ-L-lysine p-nitrophenyl ester, 2% (v/v) dimethyl sulfoxide, and 0.025 - 0.050 unit cathepsin B.

REFERENCE:  

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

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