SIGMA QUALITY CONTROL TEST PROCEDURE
Enzymatic Assay of CARBOXYPEPTIDASE B
(EC 3.4.17.2)

PRINCIPLE:
Hippuryl-L-Arg + H₂O ➞ Hippuric acid + L-Arginine

Abbreviations used:
Hippuryl-L-Arg = Hippuryl-L-Arginine

CONDITIONS: T = 25°C, pH = 7.65, A₂₅₄nm, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:
A. 25 mM Tris HCl Buffer with 100 mM Sodium Chloride, pH 7.65 at 25°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 7.65 at 25°C with 1 M NaOH.)

B. 1.0 mM Hippuryl-L-Arginine Solution (Hippuryl-L-Arg)
(Prepare 50 ml in Reagent A using Hippuryl-L-Arginine, Sigma Prod. No. H-2508. PREPARE FRESH.)

C. Carboxypeptidase B Enzyme Solution
(Immediately before use, prepare a solution containing 4 - 8 units/ml of Carboxypeptidase B in cold deionized water.)

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Hippuryl-L-Arg)</td>
<td>2.90</td>
<td>2.90</td>
</tr>
</tbody>
</table>
PROCEDURE: (continued)

Equilibrate to 25°C. Monitor the A_{254nm} until constant, using a suitably thermostatted spectrophotometer and Reagent B as the reference. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A_{254nm} for approximately 5 minutes. Obtain the \( \Delta A_{254nm}/\text{minute} \) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{254nm/\text{min Test}} - \Delta A_{254nm/\text{min Blank}}) (3)(\text{df})}{(0.36)(0.1)}
\]

3 = Total volume (in milliliters) of assay
\( \text{df} \) = Dilution factor
0.36 = Millimolar extinction coefficient of hippuric acid at 254 nm
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of hippuryl-L-arginine per minute at pH 7.65 at 25°C.
Enzymatic Assay of CARBOXYPEPTIDASE B  
EC 3.4.17.2)

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 24 mM Tris, 0.97 mM hippuryl-L-arginine, 97 mM sodium chloride, and 0.4 - 0.8 unit carboxypeptidase B.

REFERENCES:


NOTES:

1. The substrate solution has a high initial $A_{254nm}$ which requires the use of Reagent B rather than air as the reference.

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.