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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

**Enzymatic Assay of ANGIOTENSIN CONVERTING ENZYME**
**(EC 3.4.15.1)**
**Sigma Prod. No. A-6778**

**PRINCIPLE:**

Hip-L-His-L-Leu + $H_2O$ $\xrightarrow{ACE}$ Hippuric acid + L-His-L-Leu

Abbreviations used:
Hip-L-His-L-Leu = Hippuryl-L-Histidyl-L-Leucine
ACE = Angiotensin Converting Enzyme
L-His-L-Leu = L-Histidyl-L-Leucine

**CONDITIONS:** T = 37°C, pH = 8.3, $A_{228nm}$, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

A. 50 mM HEPES HCl Buffer with 300 mM Sodium Chloride, pH 8.3 at 37°C
(Prepare 100 ml in deionized water using HEPES Sodium Salt, Sigma Prod. No. H-7006, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 8.3 at 37°C with 1 M HCl.)

B. 0.3% (w/v) Hippuryl-L-Histidyl-L-Leucine Solution (HHL)
(Prepare 2 ml in Reagent A using Hippuryl-His-Leu, Free Base, Sigma Prod. No. H-1635. **PREPARE FRESH.**)

C. 1 M Hydrochloric Acid Solution (HCl)
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)

D. Ethyl Acetate
(Use Ethyl Acetate, Sigma Stock No. 27,052-0)

E. Angiotensin Converting Enzyme Solution
(Immediately before use, prepare a solution containing 0.33 unit/ml of Angiotensin Converting Enzyme in cold deionized water.)

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent B (HCl) | 0.20 | 0.20 |
| Reagent C (HCl) | ------ | 0.25 |

Mix by swirling and equilibrate to 37°C. Then add:

| | | |
|---|---|---|
| Reagent E (Enzyme Solution) | 0.05 | 0.05 |

Immediately mix by swirling and incubate for 15 minutes at 37°C. Then add:

| | | |
|---|---|---|
| Reagent C (HCl) | 0.25 | ------ |
| Reagent D (Ethyl Acetate) | 2.00 | 2.00 |

Shake vigorously for 60 seconds. Centrifuge for 2 minutes.

Pipette 1.0 ml of the clear upper layer of each vial into corresponding 4 dram vials. Place vials in a boiling water bath (approximately 1 inch depth) for 15 minutes in a hood. After the ethyl acetate has evaporated, then add:

| | | |
|---|---|---|
| Deionized Water | 3.00 | 3.00 |

Mix by inversion, but do not shake, until the residue is dissolved and transfer to suitable quartz cuvettes. Record the $A_{228nm}$[1] for both the Test and Blank, using a suitable spectrophotometer.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{228nm}\ \text{Test} - A_{228nm}\ \text{Blank})(2)(3)}{(9.8)(15)(0.91)(0.05)}$$

2 = Conversion factor since the hippuric acid detected is 1/2 of the total amount produced in the assay (2 ml of ethyl acetate is added and 1 ml of the organic layer containing the product, hippuric acid, is removed)
3 = Total volume of hippuric acid solution
9.8 = Millimolar extinction coefficient of hippuric acid at 228 nm
15 = Time (in minutes) of the assay as per the Unit Definition
0.91 = Extraction efficiency of Ethyl Acetate
0.05 = Volume (in milliliter) of enzyme used

**CALCULATIONS:** (continued)

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

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

**UNIT DEFINITION:**

One unit will produce 1.0 µmole of hippuric acid from hippuryl-his-leu per minute in 50 mM HEPES and 300 mM NaCl at pH 8.3 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 0.25 ml reaction mix, the final concentrations are 40 mM HEPES, 240 mM sodium chloride, 0.2% (w/v) hippuryl-L-histidyl-L-leucine and 0.016 unit angiotensin converting enzyme.

**REFERENCES:**

Cushman, D.W., and Cheung, H.S. (1971) *Biochem. Pharm*. **20**, 1637-1648

**NOTES:**

1. The $A_{228nm}$ (for 20 minutes) must be in the range of 0.4 - 1.0 for the Test and in the range of 0.1 - 0.15 for the Blank.

2. The assay is based on the cited reference.

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