

**Enzymatic Assay of ANGIOTENSIN CONVERTING ENZYME
(EC 3.4.15.1)**

PRINCIPLE:

Hip-L-His-L-Leu + H₂O \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.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (HHL)	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
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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
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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

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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 μ mole 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 $A_{228\text{nm}}$ (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.

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