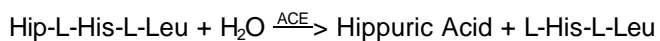


**Enzymatic Assay of  
ANGIOTENSIN CONVERTING ENZYME**

**PRINCIPLE:**



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,  $A_{214\text{nm}}$ , Light path = 1 cm

**METHOD:** HPLC Stopped Analysis of Products

**REAGENTS:**

- A. 100 mM Tris HCl Buffer with 300 mM Sodium Chloride, and 10  $\mu\text{M}$  Zinc Chloride, pH 8.3 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Sodium Chloride, Sigma Prod. No. S-9625, and Zinc Chloride, Sigma Prod. No. Z-4875. Adjust to pH 8.3 at 37°C with 1 M HCl.)
- B. 50 mM Hippuryl-L-Histidyl-L-Leucine Solution (HHL)  
(Prepare 1 ml in Reagent A using Hippuryl-His-Leu, Free Base, Sigma Prod. No. H-1635.  
**PREPARE FRESH.**)
- C. 0.02% (w/v) Hippuric Acid Standard Solution (Hipp Std)  
(Prepare 1 ml in Reagent A using Hippuric Acid, Free Acid, Sigma Prod. No. H-6375. Further dilute with Reagent A to standards containing the following concentrations (mg/ml): 0.05, 0.075, 0.10, and 0.15.)
- D. Angiotensin Converting Enzyme Solution  
(Immediately before use, prepare a solution containing 0.02-0.10 unit/ml of Angiotensin Converting Enzyme in cold Reagent A.)

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**PROCEDURE:**

Step 1:

Pipette (in milliliters) the following reagent into suitable microcentrifuge tubes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.08	0.09
Reagent B (Enz Sol)	0.01	-----

Equilibrate to 37°C. Then add:

Reagent B (HHL)	0.01	0.01
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Incubate for exactly 30 minutes at 37°C.

Terminate the reaction by heating at 100°C for 4 minutes and then microcentrifuge at 15,000 g for 10 minutes. Transfer 0.08 ml of both the Test and Blank to HPLC vials.

**Step II:**

HPLC Analysis of Products

The substrate and products are separated by reverse phase HPLC chromatography as follows:

1. Column: Supelcosil LC-18, Supeclo Prod. No. 5-8298,  
Particle size: 5 µm, 25 cm x 4.6 mm.
2.

Mobile Phase	Time (min)	0	15	25
Reagent		4.5	30	30
Reagent		95.5	70	70
3. Pressure: 2150 PSI, Flow rate 1.5 ml/min, Detection: 214nm,  
Sample Injection volume: 20 µl
4. Injection blank and then standards of Reagent C (Hipp Std) and samples. A comparison can then be made between the standard curve of hippuric acid and hippuric acid generated from the sample reaction. Convert mg of hippuric acid to µmoles.

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**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of hippuric acid})(df)(0.1)}{(0.01)(30)}$$

0.1 = Total volume (in milliliter) of enzyme assay

0.01 = Volume (in milliliter) of enzyme used

30 = Time (in minutes of assay)

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

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

**FINAL ASSAY CONDITIONS:**

In a 0.10 ml reaction mix, the final concentrations are 100 mM Tris, 300 mM Sodium Chloride, 10  $\mu\text{M}$  zinc chloride, 5 mM hippuryl-L-histidyl-L-leucine and 0.001-0.0002 unit angiotensin converting enzyme.

**UNIT DEFINITION:**

One unit will produce 1.0  $\mu\text{mole}$  of hippuric acid from hippuryl-His-Leu per min in 100 mM Tris HCl, 300 mM NaCl, and 10  $\mu\text{M}$   $\text{ZnCl}_2$  at pH 8.3 at 37°C.

**REFERENCE:**

Hooper, N.M., and Turner, A.J. (1987) Biochemical Journal **241**, 625-633

**NOTES:**

1. This assay is not to be used to assay Angiotensin Converting Enzyme, Sigma Prod. No. A-6778.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.
2. This assay is based on the cited reference.

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