SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of
ANGIOTENSIN CONVERTING ENZYME
Sigma Prod. No. A-2580

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_{214nm}

METHOD: HPLC Stopped Analysis of Products

REAGENTS:

A. 100 mM Tris HCl Buffer with 300 mM Sodium Chloride, and 10 µM 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. Acetonitrile
(Use Acetonitrile, Sigma Stock No. 27,071-7.

E. 0.08% (v/v) Phosphoric Acid, pH 2.5
(Prepare 100 ml in deionized water using Phosphoric Acid, Sigma Prod. No. P6560. The pH of a 0.08% (v/v) is approximately 2.5.)

REAGENTS: (continued)

F. Angiotensin Converting Enzyme Solution
(Immediately before use, prepare a solution containing 0.05-0.10 unit/ml of Angiotensin Converting Enzyme in cold Reagent A.)

PROCEDURE:

Step 1:

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

	Test	Blank
Reagent A (Buffer)	0.08	0.09
Reagent F (Enz Sol)	0.01	------

Equilibrate to 37°C. Then add:

Reagent B (HHL)	0.01	0.01

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, Supelco Prod. No. 5-8298,
Particle size: 5 µm, 25 cm x 4.6 mm.

2.

Mobile Phase Time (min)	0	15	25
Reagent D (Acetonitrile)	4.5	30	30
Reagent E (Phosphoric Acid)	95.5	70	70

3. Pressure: 2150 PSI^2, Flow rate 1.5 ml/min, Detection: 214nm, Sample Injection volume: 20 µl

4. Inject 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 μM zinc chloride, 5 mM Hippuryl-L-histidyl-L-leucine and 0.0005-0.001 unit angiotensin converting enzyme.

UNIT DEFINITION:

One unit will produce 1.0 μmole of hippuric acid from hippuryl-His-Leu per min in 100 mM Tris HCl, 300 mM NaCl, and 10 μM $ZnCl_2$ 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. This pressure may vary.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.
4. This assay is based on the cited reference.