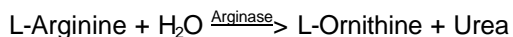


**Enzymatic Assay of ARGINASE
(EC 3.5.3.1)**

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 9.5, $A_{535\text{nm}}$, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 50 mM Manganese Maleate Activation Buffer, pH 7.0 at 37°C

The following components are made separately:

1. 100 mM Manganese Sulfate Solution
(Prepare 60 ml in deionized water using Manganese Sulfate, Sigma Prod. No. M-7634. **PREPARE FRESH.**)
2. 125 mM Maleic Acid Solution, pH 8.0 at 37°C
(Prepare 50 ml in deionized water using Maleic Acid, Sigma Prod. No. M-0375. Adjust the pH to 8.0 at 37°C using 2 M NaOH.)

Combine 50 ml of Component 1 and 40 ml of Component 2 and mix. Equilibrate to 37°C and adjust to pH 7.0 using 0.1 M HCl. Dilute to a final volume of 100 ml with deionized water.

B. 713 mM L-Arginine Substrate Solution

(Prepare 50 ml in deionized water using L-Arginine, Free Base, Sigma Prod. No. A-5006. Equilibrate to 37°C and adjust to pH 9.5 using 5 M HCl. The L-Arginine will dissolve upon the addition of the HCl. **PREPARE FRESH.**)

C. Arginase Enzyme Solution

(Prepare a solution containing 40-60 units/ml in Reagent A. Activate the enzyme by incubating for 4 hours at 37°C. Dilute 0.1 ml of activated enzyme to 50 ml using deionized water, immediately prior to use.)

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REAGENTS: (continued)

- D. 20 mM Urea Stock Solution, pH 9.5.
(Prepare 50 ml in deionized water using Urea, Sigma Prod. No. U-1250. Adjust to pH 9.5 using 0.1 N NaOH.)
- E. 4 mM Urea Standard Solution, pH 9.5.
(Prepare 10 ml by diluting 2 ml of Reagent D to 10 ml with deionized water.)
- F. BUN Acid-Color Reagent (BUN)
(Urea Nitrogen Kit, Sigma Stock No. 535-A. Immediately before use, add 60 ml of BUN Acid Reagent, Sigma Stock No. 535-3, to 40 ml of BUN Color Reagent, Sigma Stock No. 535-5, and mix.)

PROCEDURE:

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

	<u>Test</u>	<u>Test Blk</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blk</u>
Reagent C (Enz Soln)	0.30	----	----	----	----	----	----	----
Reagent E (Urea Standard Soln)	----	----	0.03	0.05	0.10	0.20	0.30	----
Deionized Water	0.30	0.30	0.57	0.55	0.50	0.40	0.30	0.60

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

Reagent B (Substrate Soln)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
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Mix by inversion and incubate at 37°C for exactly 30 minutes. Then add:

Reagent F (BUN)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Reagent C (Enz Soln)	----	0.30	----	----	----	----	----	----

Mix by inversion and place all the vials in a boiling water bath for 12 minutes. Remove and place the vials in an ice bath for 3 minutes. Transfer the solutions to suitable cuvettes and read the absorbance at 535 nm for each of the vials using deionized water as a reference.

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CALCULATIONS:

Standard Curve:

$$r A_{535\text{nm}} \text{ Standard} = A_{535\text{nm}} \text{ Standard} - A_{535\text{nm}} \text{ Standard blank}$$

Plot the $r A_{535\text{nm}}$ of the Standards vs μmoles of urea

Sample Determination:

$$r A_{535\text{nm}} \text{ Sample} = A_{535\text{nm}} \text{ Test} - A_{535\text{nm}} \text{ Test Blank}$$

Determine the μmoles of urea liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of urea liberated})(\text{df})}{(30)(0.3)}$$

df = Dilution factor

30 = Time of assay in minutes

0.3 = Volume (in milliliter) of enzyme used

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

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

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 0.03 mM manganese malate, 285 mM L-arginine and 0.024 - 0.036 unit arginase.

UNIT DEFINITION:

One unit will convert 1.0 micromole of L-arginine to ornithine and urea per minute at pH 9.5 at 37°C.

NOTES:

1. 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.