

**Determination of the Concentration and
Molecular Weight of ARGININOSUCCINIC ACID**

PRINCIPLE:

L-Argininosuccinate $\xrightarrow{\text{AL}}$ L-Arginine + Fumarate

L-Arginine $\xrightarrow{\text{Arginase}}$ Ornithine + Urea

Abbreviation used:

AL = Argininosuccinate Lyase

CONDITIONS: T = 37°C, pH = 7.5, A_{240nm}, Light path = 1 cm

METHOD: Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- B. 40 mM Potassium Sulfate Solution (K₂SO₄)
(Prepare 15 ml in deionized water using Potassium Sulfate, Sigma Prod. No. P-4042.)
- C. Argininosuccinate Acid Substrate Solution (ASA)
(Weigh approximately 2 mg of Argininosuccinic Acid and dissolve in 4.5 ml of deionized water. Then add 0.5 ml of Reagent B. Centrifuge and save the supernatant.)
- D. Arginase Enzyme Solution (Arginase)
(Immediately before use, prepare a solution containing 0.8 mg/ml of Arginase, Sigma Prod. No. A-2137, in cold deionized water.)
- E. Argininosuccinate Lyase Enzyme Solution (AL)
(Immediately before use, prepare a solution containing 20 mg/ml of Argininosuccinate Lyase, Sigma Prod. No. A-9012, in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	0.50
Reagent A (Buffer)	2.20	2.20
Reagent C (ASA)	0.50	-----
Reagent D (Arginase)	0.20	0.20

Mix by inversion and equilibrate to 37°C using a suitably thermostatted spectrophotometer. Record the initial $A_{240\text{nm}}$ for both the Test and Blank. Then add:

Reagent E (AL)	0.10	0.10
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Immediately mix by inversion and allow the reaction to proceed to completion (approximately 5 minutes). Record the final $A_{240\text{nm}}$ for both the Test and Blank.

CALCULATIONS:

$$r A = A_f - A_i$$

A_f = Final Absorbance

A_i = Initial Absorbance

$$\text{Micromoles ASA/weighed sample} = \frac{(r A_{\text{Test}} - r A_{\text{Blank}})(3)(5)}{(0.5)(2.44)}$$

ASA = Argininosuccinic Acid

3 = Total volume (in milliliters) of assay

5 = Dilution factor

0.5 = Volume (in milliliter) of argininosuccinic acid used in assay

2.44 = Millimolar extinction coefficient of fumarate at 240 nm

$$\text{Apparent Molecular Weight} = \frac{\text{mg sample weighed} \times 1000}{\text{umoles of ASA/weighed sample}}$$

1000 = Conversion factor from mg to μg

ASA = Argininosuccinic Acid

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 73 mM potassium phosphate, 0.7 mM potassium sulfate, 0.16 mg arginase, 2 mg argininosuccinate lyase, and varying amounts of argininosuccinic acid.

REFERENCE:

Havir, E.A., Hadassah, T., Ratner, S., and Warner R.C.
(1965) *Journal of Biological Chemistry* **240**, 3079-3088

NOTES:

1. This assay is based on the cited reference.
2. 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.