

**Enzymatic Assay of ARGININOSUCCINATE LYASE
(EC 4.3.2.1)**

PRINCIPLE:

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

Abbreviation used:

AL = Argininosuccinate Lyase

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

METHOD: Continuous 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 2 M KOH.)
- B. 1 M Potassium Phosphate Solution (KH₂PO₄)
(Prepare 1 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- C. 11.7 mM Argininosuccinic Acid Substrate Solution (ASA)
(Prepare 10 ml by dissolving Argininosuccinic Acid, Sodium Salt, Sigma Prod. No. A-5707, in 9.85 ml of deionized water. Add 0.15 ml of Reagent B (KH₂PO₄), mix by swirling.)
- D. Argininosuccinate Lyase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.5 units/ml of Argininosuccinate Lyase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.00	2.00
Deionized Water	0.65	0.75
Reagent D (Enzyme Solution)	0.10	-----

Mix by inversion and equilibrate to 37°C. Monitor the $A_{240\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (ASA)	0.25	0.25
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Immediately mix by inversion and record the increase in $A_{240\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{240\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{240\text{nm}}/\text{min Test} - \text{r } A_{240\text{nm}}/\text{min Blank})(3.0)(\text{df})}{(2.44)(0.1)}$$

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

2.44 = Millimolar extinction coefficient of fumarate at 240 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will form 1.0 μmole each of L-arginine and fumarate from L-argininosuccinate per minute at pH 7.5 and 37°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 68 mM potassium phosphate, 0.98 mM argininosuccinic acid, and 0.05 - 0.15 unit argininosuccinate lyase.

REFERENCE:

Ratner, S. (1970) *Methods in Enzymology*, Vol. XVIIIA, 304-309

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.