Enzymatic Assay of ADENYLOSUCCINATE LYASE
(EC 4.3.2.2)

PRINCIPLE:

Adenylosuccinic acid $\xrightarrow[\text{Lyase}]{\text{Adenylosuccinate}}$ Fumaric Acid + 5'-AMP

Abbreviation used:
5'-AMP = Adenosine 5'-Monophosphate

CONDITIONS: T = 25°C, pH = 7.0, A_{280nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer with 1 mM Ethylenediaminetetraacetic Acid (EDTA), pH 7.0 at 25°C (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic Anhydrous, Sigma Prod. No. P-5379 and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 7.0 at 25°C with 1 M KOH or 1 M NaOH.)

B. 1.72 mM Adenylosuccinic Acid (ASA)
(Prepare 10 ml in Reagent A using Adenylosuccinic Acid, Free Acid, Sigma Prod. No. A-5028. **PREPARE FRESH**.)

C. Adenylosuccinate Lyase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of Adenylosuccinate Lyase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.80	2.80
Reagent B (ASA)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{280nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Solution)	0.10	------
Reagent A (Buffer)	------	0.10

Immediately mix by inversion and record the decrease in A_{280nm} for approximately 5 minutes. Obtain the ΔA_{280nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{280nm}/\text{min Test} - \Delta A_{280nm}/\text{min Blank})(3)(df)}{(10.7)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
10.7 = Change in absorbance at 280nm of one micromole per ml
of Adenylosuccinic Acid to Fumaric Acid[1] and Adenosine 5'-Monophosphate
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 convert 1.0 µmole of adenylosuccinic acid to fumaric acid and 5'-AMP per minute at pH 7.0 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 1.0 mM ethylenediaminetetraacetic acid, 0.057 mM adenylosuccinic acid and 0.02 - 0.04 unit of adenylosuccinate lyase.

REFERENCES:

Carter C.E. and Cohen L.H. (1956) *Journal of Biological Chemistry* **222**, 17-30

Bridger, W.A. and Cohen, L.H. (1968) *Journal of Biological Chemistry* **243**, 644-650

NOTES:

1. This value is described in Carter, C.E. and Cohen, L.H. (1956).

2. This assay is based on the cited references.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.