Enzymatic Assay of L-ASPARTASE
(EC 4.3.1.1)

PRINCIPLE:

L-Aspartate $\xrightarrow{\text{L-Aspartase}}$ Fumarate + NH_3

CONDITIONS: T = 30°C, pH 8.5, A_{240nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 150 mM Tris HCl Buffer, pH 8.5 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.5 at 30°C with 1 M HCl.)

B. 60 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 1 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

C. 3.0 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 1 ml in Reagent A using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

D. 500 mM L-Aspartate Solution (L-Asp)
(Prepare 10 ml in deionized water using L-Aspartic Acid, Monopotassium, Sigma Prod. No. A-6558.)

E. 5 mM Potassium Phosphate buffer, pH 7.0 at 25°C (Enz Dil)
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M KOH.)

F. L-Aspartase Enzyme Solution
(Immediately before use, prepare a solution containing 2 units/ml of L-Aspartase in ice cold Reagent E. Place on ice.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.00	1.00
Reagent B ($MgSO_4$)	0.10	0.10
Reagent C (EDTA)	0.10	0.10
Reagent D (L-Asp)	0.30	0.30
Deionized Water	1.40	1.40

Mix by inversion and equilibrate to 30°C. Then add:

Reagent F (Enzyme Solution)	0.10	------
Reagent E (Enz Dil)	------	0.10

Immediately mix by inversion and record the increase in A_{240nm} for approximately 5 minutes. Obtain the ΔA_{240nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{240nm}/\text{min Test} - \Delta A_{240nm}/\text{min Blank})(3)(df)}{(2.53)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
2.53 = Millimolar extinction coefficient of potassium 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 convert 1.0 µmole of L-aspartate to fumarate per minute at pH 8.5 at 30°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 55 mM Tris, 2 mM magnesium sulfate, 0.1 mM ethylenediamine- tetraacetic acid, 50 mM L-aspartate, 0.2 mM potassium phosphate, and 0.2 unit L-aspartase.

REFERENCE:

Williams, V.R. and Lartigue, D.J. (1969) *Methods in Enzymology*, XIII, 354-361

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.