Enzymatic Assay of ASPARAGINASE
(EC 3.5.1.1)

PRINCIPLE:

L-Asparagine + $H_2O \xrightarrow{\text{Asparaginase}}$ L-Aspartate + NH_3

CONDITIONS: T = 37°C, pH = 8.6, A_{436nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Tris Buffer, pH 8.6 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.6 at 37°C with 1 M HCl.)

B. 189 mM L-Asparagine Solution
(Prepare 10 ml in deionized water using L-Asparagine, Anhydrous, Prod. No. A-0884.)

C. 6 mM Ammonium Sulfate Standard Solution ($(NH_4)_2SO_4$ Std)
(Prepare 100 ml deionized water using Ammonium Sulfate, Grade I, Prod. No. A-5132.)

D. 1.5 M Trichloroacetic Acid (TCA)
(Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, Stock No. 490-10.)

E. Ammonia Color Reagent
(Use Nessler's Reagent, Aldrich Stock No. 34,518-8.)

F. Asparaginase Enzyme Solution
(Immediately before use, prepare a solution containing 2.0 - 4.0 units/ml of Asparaginase in cold deionized water.)

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PROCEDURES:

Step 1:

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

	Test	Blank	Std 1	Std 2		Std 3	Blank
Reagent A (Buffer)	1.00	1.00	1.00	1.00	1.00	1.00	
Reagent B (L-Asparagine Soln)	0.10	0.10	----	----	----	----	
Reagent C ($(NH_4)_2SO_4$ Std)	----	----	0.25	0.50	1.00	----	
Deionized Water	0.90	0.90	0.85	0.60	0.10	1.10	

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.10	----	----	----	----	----	

Immediately mix by inversion and incubate at 37°C for 30 minutes. Then add:

Reagent D (TCA)	0.10	0.10	0.10	0.10	0.10	0.10	
Reagent F (Enzyme Solution)	----	0.10	----	----	----	----	

Mix by inversion. Centrifuge for 2 minutes to clarify.

Step 2:

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

Deionized Water	4.30	4.30	4.30	4.30	4.30	4.30	
Supernatant (from Step 1)	0.20	0.20	0.20	0.20	0.20	0.20	
Reagent E (Ammonia Color Reagent)	0.50	0.50	0.50	0.50	0.50	0.50	

Immediately mix by inversion and after 1 minute record the A_{436nm} for Standards, Tests, and Blanks.

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CALCULATIONS:

Standard Curve:

ΔA_{436nm} Standard = A_{436nm} Standard - A_{436nm} Standard Blank

Prepare a standard curve by plotting the ΔA_{436nm} of the Standard versus Ammonia (NH_3) concentration. Note that 1 mole of Ammonium Sulfate corresponds to 2 moles of Ammonia, therefore a 6 mM Ammonium Sulfate standard is equivalent to a 12 mM ammonium standard.

Sample Determination:

ΔA_{436nm} Test = A_{436nm} Test - A_{436nm} Test Blank

Determine the µmoles of NH_3 liberated using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole of } NH_3 \text{ liberated})(2.20)}{(0.2)(30)(0.1)}$$

2.20 = Volume of Step 1
0.2 = Volume of Step 1 used in Step 2
30 = Time of assay in minutes
0.1 = Volume (in milliliters) 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 liberate 1.0 µmole of ammonia from L-asparagine per minute at pH 8.6 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.20 ml reaction mix, the final concentrations are, 23 mM Tris, 8.6 mM L-asparagine and 0.2 - 0.4 units of asparaginase.

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REFERENCES:

Shirfrin, S., Parrott, C.L. and Luborsky, S.W. (1974) *Journal of Biological Chemistry* **249**, 1335-1340

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.