

SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation**Enzymatic Assay of ASPARAGINASE
(EC 3.5.1.1)****PRINCIPLE:**

L-Asparagine + H₂O $\xrightarrow{\text{Asparaginase}}$ L-Aspartate + NH₃

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 ((NH₄)₂SO₄ 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:

| | <u>Test</u> | <u>Blank</u> | <u>Std 1</u> | <u>Std 2</u> | <u>Std 3</u> | <u>Std Blank</u> |
|--|-------------|--------------|--------------|--------------|--------------|----------------------|
| 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) ₂ SO 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:

$$\Delta A_{436\text{nm}} \text{ Standard} = A_{436\text{nm}} \text{ Standard} - A_{436\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting the $\Delta A_{436\text{nm}}$ 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:

$$\Delta A_{436\text{nm}} \text{ Test} = A_{436\text{nm}} \text{ Test} - A_{436\text{nm}} \text{ Test Blank}$$

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.

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