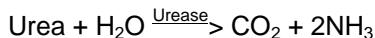

**SIGMA QUALITY CONTROL TEST
PROCEDURE****Product Information****Enzymatic Assay of UREASE¹
from Jack Beans
(EC 3.5.1.5)****PRINCIPLE:****CONDITIONS:** T = 25°C, pH = 7.0**METHOD:** Titrimetric**REAGENTS:**

- A. 750 mM Sodium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 200 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.0 at 25°C with 1 M NaOH. Store at room temperature.)
- B. 500 mM Urea with 0.05% (w/v) Bovine Serum Albumin Solution (Urea)
(Prepare 50 ml in Reagent A using Urea, Sigma Prod. No. U-1250, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.0 at 25°C with 100 mM HCl or 100 mM NaOH, if necessary.)
- C. 0.10% (w/v) 3-(4-Dimethylamino-1-Naphthylazo)-4-Methoxybenzenesulfonic Acid Solution (Indicator)
(Prepare 100 ml in deionized water using 3-(4-Dimethylamino-1-Naphthylazo)-4-Methoxybenzenesulfonic Acid Solution, Sigma Prod. No. D-5407. Facilitate dissolving by first adding 2.6 ml of 100 mM NaOH then adjusting the final volume to 100 ml with deionized water.)
- D. 100 mM Standardized Hydrochloric Acid Solution (HCl)
(Prepare 100 ml in cold deionized water using Concentrated Hydrochloric Acid, Sigma Prod. No. H-7020. Standardize according to the ACS Reagent Procedure.²)
- E. 20 mM Sodium Phosphate Buffer, pH 7.0 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.0 at 25°C with 1 M NaOH.)

**Enzymatic Assay of UREASE¹
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REAGENTS: (continued)

F. Urease Enzyme Solution
(Immediately before use, prepare a solution containing 200 - 400 units/ml of Urease in cold Reagent E.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Urea)	1.00	1.00

Equilibrate to 25°C. Then add:

Reagent F (Enzyme Solution)	0.10	-----
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Mix by stirring and incubate at 25°C for exactly 5 minutes. Then add:

Reagent D (HCl)	3.00	3.00
Reagent F (Enzyme Solution)	-----	0.10
Reagent C (Indicator)	2 drops	2 drops

Using a suitable magnetic stirrer, titrate immediately with Reagent D (HCl) by adding small amounts until the color of the indicator turns from orange to pink. This is the endpoint. Record the volume (vol.) of Reagent D (HCl) used for both the Test and Blank solutions.

CALCULATION:

$$\text{Vol. HCl} = \text{Vol. HCl}_{\text{Test}} - \text{Vol. HCl}_{\text{Blank}}$$

$$\text{Units/ml enzyme} = \frac{(\text{HCl Molarity})(\text{Vol. HCl})(1000)(\text{df})}{(5)(0.1)}$$

Vol. HCl = Volume (in milliliters) of Reagent D used in the titration

1000 = Conversion factor from millimoles to micromoles as per the Unit definition

df = Dilution factor

5 = Time of assay (in minutes) as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used

**Enzymatic Assay of UREASE¹
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CALCULATION: (continued)

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

$$\text{Units/g solid} = \text{Units/mg solid} \times 1000$$

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of ammonia from urea per minute at pH 7.0 at 25°C, under our assay conditions. It is equivalent to 1.0 I.U. or 0.054 Sumner unit (1.0 mg ammonia nitrogen in 5 minutes at pH 7.0 at 20°C).

FINAL ASSAY CONCENTRATIONS:

In a 1.10 ml reaction mix, the final concentrations are 684 mM sodium phosphate, 455 mM urea, 0.05% (w/v) bovine serum albumin and 25 - 50 units urease.

REFERENCE:

(1993) *Reagent Chemicals ACS Specifications* 8th edition, 91

Worthington, C.E. (1972) *Worthington Enzyme Manual*, pp. 146-148, Worthington Biochemical Corporation, Freehold, NJ

NOTES:

1. This assay is not to be used to determine the activity of Urease, from *Bacillus pasteurii*, Sigma Prod. No. U-7127.
2. The standardization of HCl is described in (1993) *Reagent Chemicals ACS Specifications*.
3. This assay is based on the cited references.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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