SIGMA

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of UREASE[1] from Jack Beans (EC 3.5.1.5)

PRINCIPLE:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

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.)

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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:

	Test	Blank
Reagent B (Urea)	1.00	1.00

Equilibrate to 25°C. Then add:

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

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

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CALCULATION: (continued)

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

Units/g solid = Units/mg solid x 1000

UNIT DEFINITION:

One unit will liberate 1.0 µmole 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.