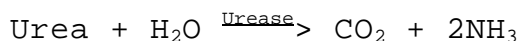


**Enzymatic Assay of UREASE**  
**(EC 3.5.1.5)**

**PRINCIPLE:**



**CONDITIONS:** T = 30°C, pH = 8.2, A<sub>480nm</sub>, Light Path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 10 mM Potassium Phosphate Buffer, pH 8.2 at 30°C with 10 mM Lithium Chloride and 1 mM Ethylenediaminetetraacetic Acid.  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504, Lithium Chloride, Anhydrous, Sigma Prod. No. L-0505, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4S. Adjust to pH 8.2 at 30°C with 1 M HCl.)
- B. 66 mM Urea Solution.  
(Prepare 25 ml in Reagent A using Urea, Sigma Prod. No. U-1250.)
- C. 0.4% (w/v) Ficoll Solution  
(Prepare 20 ml in deionized water using Ficoll, Type 400-DL, Sigma Prod. No. F-9378.)
- D. Nessler's Color Reagent (NCR)  
(Prepare 75 ml using 15 ml Aldrich Nessler's Reagent, Aldrich Stock No. 34,514-8, 15 ml Reagent C, and 45 ml deionized water.)
- E. 2.50 mM Ammonia Standard Solution (Amm Std)  
(Prepare 50 ml in deionized water using Ammonium Sulfate, Sigma Product No. A-2939.)
- F. Urease Enzyme Solution  
(Immediately before use, prepare a solution containing 100 - 150 units/ml of Urease in cold Reagent A.)

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**PROCEDURE:**

Pipette (in milliliters) the following reagents into a suitable container:

	<u>Test</u>
Reagent B (Urea Solution)	10.00

Incubate at 30°C. Then at time zero add:

Reagent F (Enzyme Solution)	0.025
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Immediately mix by inversion and remove 1.0 ml aliquot from the Test solution. Transfer to a suitable container containing 5.0 ml of Reagent D (NCR) and mix by inversion. This is to be used as the Reagent Blank. Continue to incubate at 30°C. Then at 2, 4, and 6 minutes (time increments), transfer 1.0 ml Test solution into suitable containers containing 5.0 ml of Reagent D (NCR) and mix by inversion. Transfer the solutions to suitable cuvetts and record the  $A_{480\text{nm}}$  for the Reagent Blank and Tests using a suitable spectrophotometer.

**COLORIMETRIC:**

Standards:

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

	<u>Std</u>	<u>Blank</u>	<u>Std1</u>	<u>Std2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std 6</u>
Deionized water	1.00	0.90	0.80	0.60	0.40	0.20	-----	
Reagent E (Amm Std)	-----	0.10	0.20	0.40	0.60	0.80	1.00	
Reagent D (NCR)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

Mix by inversion. Transfer to suitable cuvetts, measure and record the  $A_{480\text{nm}}$  for the Standard Blank and Standards.

**CALCULATIONS:**

$$r A_{480\text{nm}} \text{ Standard} = A_{480\text{nm}} \text{ Standard} - A_{480\text{nm}} \text{ Standard Blank}$$

Plot  $\mu\text{moles of NH}_3$  vs  $r A_{480\text{nm}}$  to obtain  $r A_{480\text{nm}}/\mu\text{mole NH}_3$

$$r A_{480\text{nm}} \text{ Test} = A_{480\text{nm}} \text{ Test} - A_{480\text{nm}} \text{ Reagent Blank}$$

$$r A_{480\text{nm}} \text{ Test/min} = r A_{480\text{nm}} \text{ Test/Time increment (in minutes)}$$

**Enzymatic Assay of Urease  
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**CALCULATIONS:** (continued)

$$\text{Units/g enzyme} = \frac{r A_{480\text{nm}} \text{ Test/minute} (1000)}{(r A_{480\text{nm}}/\mu\text{mole NH}_3)(\text{mg enzyme/ml RM})}$$

RM = Reaction mixture

1000 = Conversion factor for converting milligrams to grams

**UNIT DEFINITION:**

One unit will liberate 1.0  $\mu\text{mole}$  of  $\text{NH}_3$  from urea per minute at pH 8.2 at 30°C.

**FINAL ASSAY CONDITIONS:**

In a 10.025 ml reaction mixture the final concentrations are 10 mM potassium phosphate, 10 mM lithium chloride, 1 mM ethylenediaminetetraacetic acid, 66 mM urea and 2.5-3.75 units urease.

**NOTES:**

1. 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.**