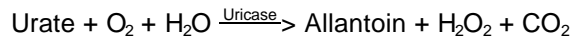


## Enzymatic Assay of URICASE<sup>1</sup> (EC 1.7.3.3)

### PRINCIPLE:



**CONDITIONS:** T = 25°C, pH = 8.5, A<sub>293nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 20 mM Borax Solution  
(Prepare 100 ml in deionized water using Borax, Sodium Tetraborate, Sigma Prod. No. B-9876.)
- B. 20 mM Boric Acid Buffer, pH 8.5 at 25°C  
(Prepare 200 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 8.5 at 25°C with Reagent A.)
- C. 48 μM Uric Acid Solution (Uric Acid)  
(Prepare 125 ml in Reagent B using Uric Acid Sodium Salt, Sigma Prod. No. U-2875. The solution may require heat to effect complete dissolution. The A<sub>293nm</sub> of this solution should be from 0.6 - 0.7 in a 1 cm light path. Dilute accordingly with Reagent B. **PREPARE FRESH.**)
- D. Uricase Enzyme Solution  
(Immediately before use, prepare a solution containing approximately 0.25 unit/ml of Uricase in Reagent B.)

**Enzymatic Assay of URICASE<sup>1</sup>**  
**(EC 1.7.3.3)**

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent C (Uric Acid)	2.90	2.90

Equilibrate to 25°C. Monitor the  $A_{293nm}$  until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	-----
Reagent B (Buffer)	-----	0.10

Immediately mix by inversion and record the decrease in  $A_{293nm}$  for approximately 5 minutes. Obtain the  $\Delta A_{293nm}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{293nm}/\text{min Test} - \Delta A_{293nm}/\text{min Blank})(3)(df)}{(12.3)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

12.3 = Millimolar extinction coefficient of Uric Acid at 293nm

0.1 = Volume (in milliliter) 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 convert 1.0  $\mu\text{mole}$  of uric acid to allantoin per minute at pH 8.5 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 20 mM boric acid, 46  $\mu\text{M}$  uric acid, and 0.025 unit uricase.

**Enzymatic Assay of URICASE<sup>1</sup>**  
**(EC 1.7.3.3)**

**REFERENCE:**

Mahler, H.R., Hübscher, G., and Baum, H. (1955) *Journal of Biological Chemistry* **216**, 625-641

**NOTES:**

1. This assay is not to be used to assay the activity of Uricase, Microbial source, from *Arthrobacter globiformis*, Sigma Prod. No. U-7128.)
2. This assay is based on the cited reference.
3. 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.**