Enzymatic Assay of URICASE[1]
(EC 1.7.3.3)

PRINCIPLE:

Urate + O_2 + H_2O $\xrightarrow{\text{Uricase}}$ Allantoin + H_2O_2 + CO_2

CONDITIONS: T = 25°C, pH = 8.5, A_{293nm}, 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 25EC
(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_{293nm} 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.)

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PROCEDURE:

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

	Test	Blank
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 ΔA_{293nm}/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 μmole 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 μM uric acid, and 0.025 unit uricase.

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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.