Enzymatic Assay of URICASE
(EC 1.7.3.3)

PRINCIPLE:

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

CONDITIONS: T = 25°C, pH = 9.0, A_{293nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 20 mM Boric Acid Buffer, pH 9.0 at 25°C
(Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 9.0 at 25°C with 1 M Hcl or 1 M NaOH.)

B. 3.57 mM Uric Acid Solution (Urate)
(Prepare 10 ml in Reagent A using Uric Acid, Sodium Salt, Sigma Prod. No. U-2875. The solution may require heat and vortexing in order to effect complete dissolution.)

C. Uricase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Uricase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	3.00	3.00
Reagent B (Urate)	0.10	0.10

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PROCEDURE: (continued)

Mix by swirling and equilibrate to 25°C. Monitor the A_{293nm} until constant using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent C (Enzyme Solution)	0.02	------
Reagent A (Buffer)	------	0.02

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.[1]

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{293nm}/\text{min Test} - \Delta A_{293nm}/\text{min Blank})(3.12)(df)}{(12.6)(0.02)}$$

3.12 = Total volume (in milliliters) of assay
df = Dilution factor
12.6 = Millimolar extinction coefficient[1] of Uric Acid at 293nm
0.02 = 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 9.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.12 ml reaction mix, the final concentrations are 20 mM boric acid, 0.11 mM uric acid, and 0.01 - 0.02 unit uricase.

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REFERENCE:

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

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) 2nd ed.,
Volume I, 518, Academic Press, New York, NY

NOTES:

1. If the ΔA293 nm/min is 70.06, prepare a fresh dilutions of the enzyme with a higher dilution factor so that the ΔA293 nm/min is <0.06.

2. The millimolar extinction coefficient of Urate is described in Bergmeyer, H.U. et al. (1974).

3. This assay is based on the cited references.

4. 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.