Enzymatic Assay of XANTHINE OXIDASE
(EC 1.1.3.22)

PRINCIPLE:

$$\text{Xanthine} + H_2O + O_2 \xrightarrow{\text{Xanthine Oxidase}} \text{Uric Acid} + H_2O_2$$

CONDITIONS: T = 25°C, pH = 7.5, A_{290nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 25°C with 1 M KOH.)

B. 0.15 mM Xanthine Solution
(Prepare 100 ml by initially dissolving Xanthine, Sigma Prod. No. X-0626, in a minimal volume of NaOH. Add approximately 90 ml of deionized water. Adjust to pH 7.5 at 25°C with either 1 M NaOH or 1 M HCl. Dilute to a final volume of 100 ml. **PREPARE FRESH**.)

C. Xanthine Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Xanthine Oxidase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.90	1.90
Reagent B (Xanthine)	1.00	1.00
Deionized Water	------	0.10

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the A_{290nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent C (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{290nm} for approximately 5 minutes. Obtain the ΔA_{290nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{290nm}/\text{min Test} - \Delta A_{290nm}/\text{min Blank})(3)(df)}{(12.2)\ (0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
12.2 = Millimolar extinction coefficient of Uric Acid at 290 nm
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 xanthine to uric acid per minute at pH 7.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 33 mM potassium phosphate, 0.050 mM xanthine and 0.01 - 0.02 unit xanthine oxidase.

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REFERENCE:

Bergmeyer, H.U., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Second Edition, Volume I, 521-522, Academic Press Inc., New York, NY

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.