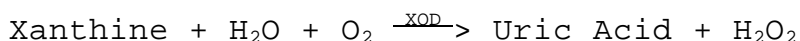
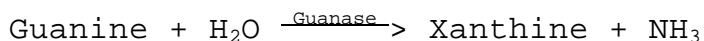


**Enzymatic Assay of GUANASE
(EC 3.5.4.3)**

PRINCIPLE:



CONDITIONS: T = 25°C, pH = 8.0, A_{290nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 25°C with 1 M HCl.)
- B. 0.001% (w/v) Guanine Solution (Guanine)
(Prepare by dissolving 10 mg of Guanine, Sigma Prod. No. G-0381, in 10 ml of 1 M NaOH. Then add 1 ml of the Guanine Solution to 100 ml of Reagent A. Adjust to pH 8.0 at 25°C with either 1 M HCl or 1 M NaOH, if necessary.)
- C. Xanthine Oxidase Enzyme Solution (XOD)
(Prepare a solution containing 0.3 unit/ml of Xanthine Oxidase, Sigma Prod. No. X-1875, in cold deionized water.)
- D. Guanase Enzyme Solution (Guanase)
(Immediately before use, prepare a solution containing 0.10 - 0.15 unit/ml of Guanase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Guanine)	2.80	2.80
Reagent C (XOD)	0.10	0.10

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PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent D (Guanase)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the increase in $A_{290\text{nm}}$ for approximately 10 minutes. Obtain the $r A_{290\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

12.2 = Millimolar extinction coefficient¹ of Uric Acid
at 290 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will deaminate 1.0 μmole of guanine to xanthine per minute at pH 8.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 93 mM Tris, 0.0009% (w/v) guanine, 62 mM sodium hydroxide, 0.03 unit xanthine oxidase, and 0.01 - 0.015 unit guanase.

REFERENCES:

Kalckar, H.M. (1947) *Journal of Biological Chemistry* **167**, 461-475

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

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NOTES:

1. The millimolar extinction coefficient was experimentally determined by Sigma.
2. Xanthine Oxidase Unit Definition: One unit will convert 1.0 μ mole of xanthine to uric acid per minute at pH 7.5 at 25°C.
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.