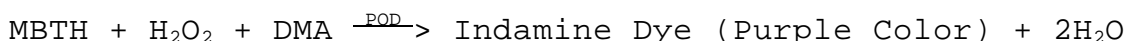
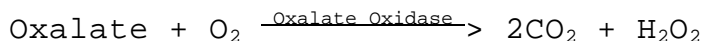


**Enzymatic Assay of OXALATE OXIDASE
(EC 1.2.3.4)**

PRINCIPLE:



Abbreviations used:

MBTH = 3-Methyl-2-Benzothiazolinone Hydrazone

DMA = N,N-Dimethylaniline

POD = Peroxidase

CONDITIONS: T = 37°C, pH = 3.8, A_{600nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Succinate buffer, with 0.79 mM N,N-Dimethylaniline and 0.11 mM 3-Methyl-2-Benzothiazolinone Hydrazone, pH 3.8 at 37°C (MBTH) (Prepare 100 ml in deionized water using Succinic Acid, Sigma Prod. No. S-7501, 3-Methyl-2-Benzothiazolinone Hydrazone, Hydrochloride, Sigma Prod. No. M-8006, and N,N-Dimethylaniline, Sigma Prod. No. D-8509. Adjust to pH 3.8 at 37°C with 1 M NaOH.)
- B. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA) (Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- C. 200 mM Oxalic Acid Solution, pH 3.8 at 37°C (Oxalic Acid) (Prepare 1 ml in deionized water using Oxalic Acid, Free Acid, Sigma Prod. No. O-0376. Adjust to pH 3.8 at 37°C with 1 M NaOH.)
- D. Peroxidase Enzyme Solution (POD) (Immediately before use, prepare a solution containing 1 mg/ml of Peroxidase, Sigma Prod. No. P-8375, in cold deionized water.)

**Enzymatic Assay of OXALATE OXIDASE
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REAGENTS: (continued)

E. Oxalate Oxidase Enzyme Solution (Ox Oxidase)
(Immediately before use, prepare a solution containing
0.25 - 0.50 unit/ml of Oxalate Oxidase in cold
deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (MBTH)	2.70	2.70
Reagent B (EDTA)	0.10	0.10
Deionized Water	0.10	0.10
Reagent C (Oxalic Acid)		0.02
		0.02
Reagent D (POD)	0.01	0.01

Mix by inversion and equilibrate to 37°C. Monitor the
A_{600nm} until constant, using a suitably thermostatted
spectrophotometer. Then add:

Reagent E (Ox Oxidase)	0.02	-----
Deionized Water	-----	0.02

Immediately mix by inversion and record the increase in
A_{600nm} for approximately 5 minutes. Obtain the r A₆₀₀/minute
using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{600\text{nm}}/\text{min Test} - r A_{600\text{nm}}/\text{min Blank})(2.95)(df)}{(26.4)(0.02)}$$

2.95 = Total volume (in milliliters) of assay

df = Dilution factor

26.4 = Millimolar extinction coefficient of Indamine dye
at

600 nm

0.02 = Volume (in milliliter) of Oxalate Oxidase used

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

**Enzymatic Assay of OXALATE OXIDASE
(EC 1.2.3.4)**

UNIT DEFINITION:

One unit will form 1.0 μ mole of H_2O_2 from oxalate per minute at pH 3.8 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 2.95 ml reaction mix, the final concentrations are 46 mM succinic acid, 0.1 mM 3-methyl-2-benzothiazolinone hydrazone, 0.72 mM N,N-dimethylaniline, 3.4 mM ethylenediaminetetraacetic acid, 1 mM oxalic acid, 0.01 mg peroxidase, and 0.005 - 0.01 unit oxalate oxidase.

REFERENCE:

Laker, M.F., Hoffman, A.F., and Meeuse, J.D. (1980)
Clinical Chemistry **26**, 827-830

NOTES:

1. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.
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.