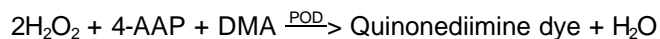
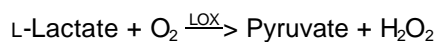


Enzymatic Assay of LACTATE OXIDASE

PRINCIPLE:



Abbreviations used:

LOX = L-Lactate Oxidase

4-AAP = Aminoantipyrine

DMA = N,N-Dimethylaniline

POD = Peroxidase

CONDITIONS: T = 37°C, pH = 6.5, $A_{565\text{nm}}$, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 200 mM 3,3-Dimethylglutaric Acid-NaOH Buffer, pH 6.5 at 37EC (DMGA)
(Prepare 5 ml in deionized water using 3,3-Dimethylglutaric Acid, Sigma Prod. No. D-4379.
Adjust to pH 6.5 at 37°C with 1 M NaOH.)
- B. 15 mM 4-Aminoantipyrine Solution (4-AAP)
(Prepare 1 ml in deionized water using 4-Aminoantipyrine, Free Base, Sigma Prod.
No. A-4382.)
- C. 500 mM L(+)Lactic Acid Solution, pH 6.5 at 37EC (Lactic Acid)
(Prepare 1 ml in deionized water using L(+)Lactic Acid, Free Acid, Sigma Prod. No. L-1750.
Adjust to pH 6.5 with 1 M NaOH.)
- D. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 50 Purpurogallin units/ml of Peroxidase
Type II from Horseradish, Sigma Prod. No. P-8250, in cold deionized water.)

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REAGENTS: (continued)

- E. 10 mM Potassium Phosphate Buffer with 0.010 mM Flavin Adenine Dinucleotide (FAD), pH 7.0 at 37°C (Enzyme Diluent)
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Flavin Adenine Dinucleotide, Disodium Salt, Sigma Prod. No. F-6625. Adjust to pH 7.0 at 37°C with 1 M NaOH. **PREPARE FRESH.**)
- G. 0.2% (v/v) N,N-Dimethylaniline Solution (DMA)
(Prepare 10 ml in deionized water using N,N-Dimethylaniline, Sigma Prod. No. D-8509.)
- H. 0.25% (w/v) Dodecylbenzenesulfonic Acid Solution (DBS)
(Prepare 5 ml in deionized water using Dodecylbenzenesulfonic Acid, Sodium Salt, Sigma Prod. No. D-2525.)
- I. Lactate Oxidase Enzyme Solution (LOX)
(Immediately before use, prepare a solution containing 0.1 - 0.2 units/ml of Lactate Oxidase in cold Reagent E.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container.

| | | |
|-------------------------|------|------|
| Reagent A (DMGA) | 2.00 | |
| Reagent D (POD) | | 1.00 |
| Reagent B (4-AAP) | | 1.00 |
| Reagent C (Lactic Acid) | | 1.00 |
| Deionized Water | | 3.00 |

Mix by inversion and equilibrate to 37°C.

Pipette (in milliliters) the following reagents into a suitable cuvette:

| | <u>Test</u> | <u>Blank</u> |
|-------------------|-------------|--------------|
| Reaction Cocktail | 0.80 | 0.80 |
| Reagent G (DMA) | 0.20 | 0.20 |

Mix by inversion and equilibrate to 37°C. Then add:

| | | |
|----------------------------|-------|-------|
| Reagent I (LOX) | 0.020 | ----- |
| Reagent E (Enzyme Diluent) | ----- | 0.020 |

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PROCEDURE: (continued)

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

| | <u>Test</u> | <u>Blank</u> |
|-----------------|-------------|--------------|
| Reagent H (DBS) | 2.00 | 2.00 |

Mix by inversion and record the $A_{565\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(A_{565\text{nm}} \text{ Test} - A_{565\text{nm}} \text{ Blank})(3.02)(\text{df})}{(35.33)(0.5)(10)(0.02)}$$

3.02 = Total volume of assay

df = Dilution factor

35.33 = Millimolar extinction coefficient of Quinonediimine dye at 565 nm.

0.5 = Conversion factor based on one mole of H_2O_2 produces half a mole of Quinonediimine dye

10 = Time of assay (in minutes) as per unit definition

0.02 = Volume (in milliliter) of enzyme used

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

FINAL ASSAY CONCENTRATIONS:

In a 1.02 ml reaction mix, the final concentrations are 39 mM 3,3 dimethylglutaric acid, 5 units peroxidase, 1.5 mM 4-aminoantipyrine, 49 mM L(+)lactic acid, 0.04% (v/v) N,N-dimethylaniline, 0.20 mM potassium phosphate, 0.20 μM FAD and 0.002 - 0.004 unit lactate oxidase.

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of L-lactate to pyruvate and H_2O_2 per minute at pH 6.5 at 37°C.

REFERENCES:

Lockridge, O., Massey, V., and Sullivan, P.A., (1972) *Journal of Biological Chemistry* **247**, 8097-8106.

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NOTES:

1. Unit Definition for Peroxidase: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C. This purpurogallin (20 seconds) unit is equivalent to approximately 18 μ M units per minute at 25°C.
2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.