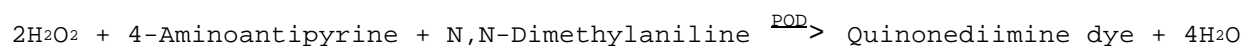
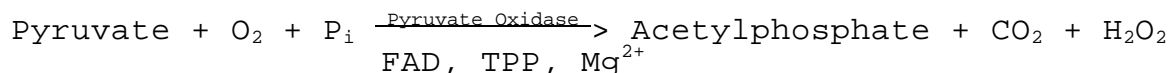


**Enzymatic Assay of PYRUVATE OXIDASE
(EC 1.2.3.3)
from *Pediococcus* species**

PRINCIPLE:



Abbreviations used:

P_i = Inorganic Phosphate

FAD = Flavin Adenine Dinucleotide

TPP = Thiamine Pyrophosphate

POD = Peroxidase

CONDITIONS: T = 37°C, pH = 6.7, A_{565nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 1 M Potassium Phosphate Buffer, pH 6.7 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.7 at 37°C with 1 M NaOH.)
- B. 1 mM Flavin Adenine Dinucleotide Solution (FAD)
(Prepare 1 ml in deionized water using Flavin Adenine Dinucleotide, Disodium Salt, Sigma Prod. No. F-6625.
Prepare Fresh.)
- C. 10 mM Cocarboxylase (Thiamine Pyrophosphate) Solution (TPP)
(Prepare 1 ml in deionized water using Cocarboxylase, Sigma Prod. No. C-8754. **PREPARE FRESH.**)
- D. 15 mM 4-Aminoantipyrine Solution (4-AAP)
(Prepare 100 ml in deionized water using 4-Aminoantipyrine Free Base, Sigma Prod. No. A-4382.)
- E. Peroxidase Enzyme Solution (POD)
Immediately before use, prepare a solution containing 50 purpurogallin units/ml in deionized water using Peroxidase, Sigma Prod. No. P-8250.)

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REAGENTS: (continued)

- F. 100 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- G. 0.2% (v/v) N,N-Dimethylaniline Suspension (DMA)
(Prepare 100 ml in deionized water using N,N-Dimethylaniline, Sigma Prod. No. D-8509. N,N-Dimethylaniline is insoluble in deionized water. Prepare by adding 0.2 ml of N,N-Dimethylaniline to 80 ml of deionized water. Mix by swirling and bring to a total volume of 100 ml with deionized water.)
- H. Reaction Mixture II (Rxn II)
(Prepare by combining 1 ml of Reagent F and 2 ml of Reagent G. **PREPARE FRESH.**)
- I. 1 M Sodium Pyruvate Solution (Pyr)
(Prepare 1 ml in deionized water using Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256.)
- J. 200 mM Sodium Phosphate Solution
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0876.)
- K. 100 mM Citric Acid Solution
(Prepare 100 ml in deionized water using Citric Acid, Monohydrate, Sigma Prod. No. C-7129.)
- L. McIlvain Buffer
(Prepare 100 ml by adding 63.15 ml of Reagent J and 36.85 ml of Reagent K.)
- M. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 100 ml in Reagent L using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.5 with either 1 M NaOH or 1 M HCl.)
- N. 10 mM Potassium Phosphate with 10 μ M Flavin Adenine Dinucleotide Solution, pH 7.0 at 37°C (Enzyme Diluent)
(Prepare 100 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 KOH.)

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REAGENTS: (continued)

- O. Pyruvate Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing
0.1 - 0.2 unit/ml of Pyruvate Oxidase in Reagent N.)

PROCEDURE:

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

Deionized Water	1.70
Reagent A (Buffer)	2.00
Reagent B (FAD)	0.10
Reagent C (TPP)	0.20
Reagent D (4-AAP)	1.00
Reagent E (POD)	1.00

Mix by swirling and equilibrate to 37°C.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	0.60	0.60
Reagent H (Rxn II)	0.30	0.30
Reagent I (Pyr)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the
 $A_{565\text{nm}}$ until constant, using a suitably thermostatted
spectrophotometer. Then add:

Reagent N (Enzyme Diluent)	-----	0.02
Reagent O (Enzyme Solution)	0.02	-----

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

Reagent M (EDTA)	2.00	2.00
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Mix by inversion and incubate at 25°C for exactly 5
minutes. Transfer to suitable cuvettes and record the $A_{565\text{nm}}$
for both the Test and Blank using a suitable
spectrophotometer.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{565\text{nm}} \text{ Test} - A_{565\text{nm}} \text{ Blank})(2)(3.02)(\text{df})}{(10)(23.56)(0.02)}$$

3.02 = Total volume (in milliliters) of assay

2 = 2 moles H₂O₂ used per mole of dye

10 = Time (in minutes) of assay as per the
Unit Definition

23.56 = Millimolar extinction coefficient of
quinonediimine

dye at 565 nm

0.02 = Volume (in milliliters) 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 produce 1.0 μmole of H₂O₂ per minute during the conversion of pyruvate and phosphate to acetylphosphate and CO₂.

FINAL ASSAY CONCENTRATION:

In a 1.02 ml reaction mix, the final concentrations are 196 mM potassium phosphate, 0.01 mM flavin adenine dinucleotide, 0.20 mM cocarboxylase, 1.5 mM 4-aminoantipyrine, 0.04% (v/v) N,N-dimethylaniline, 9.8 mM magnesium chloride, 5 purpurogallin units peroxidase and 0.05 - 0.1 unit pyruvate oxidase.

REFERENCE:

Toyo Jozo Co. Ltd., *Enzyme Handbook*, pp 23, June 1982, Tokyo, Japan (Jozo)

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.

**Enzymatic Assay of PYRUVATE OXIDASE
(EC 1.2.3.3)
from *Pediococcus* species**

NOTES: (continued)

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.