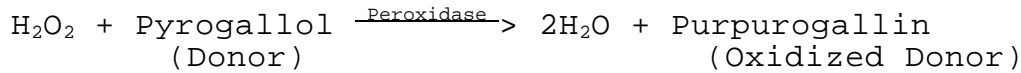


Enzymatic Assay of PEROXIDASE, INSOLUBLE¹

PRINCIPLE:



CONDITIONS: T = 30°C, pH 6.0, A_{420nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 6.0 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.0 at 30°C with 1 M KOH.)
- B. 0.50% (w/w) Hydrogen Peroxide Solution (H₂O₂)
(Prepare 50 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009. **PREPARE FRESH.**)
- C. 5.0% (w/v) Pyrogallol Solution (Pyrogallol)
(Prepare 50 ml in deionized water using Pyrogallol, Sigma Prod. No. P-0381. **PREPARE FRESH AND KEEP FROM LIGHT.**)
- D. Peroxidase Insoluble Enzyme Suspension (Insol Enz)
(Place sample on a small Buchner funnel. Using a suction flask and a light rate of suction, wash the agarose resin with about 10 times the sample volume of deionized water. Dry the moist gel with suction until the top of the packed gel cracks. Weigh the moist gel and place it into an appropriate container. Add one ml of deionized water per mg of sample gel. Immediately prior to assay dilute 0.1 ml of gel suspension to 2.1 ml with cold deionized water.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into a suitable container while continuously mixing with a magnetic stirrer.

	<u>Test</u>
Reagent A (Buffer)	3.30
Reagent B (H ₂ O ₂)	1.70
Reagent C (Pyrogallol)	3.30
Deionized Water	21.70

Equilibrate to 30°C. Then add:

Reagent D (Insol Enz)	0.10
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Immediately filter enough of the reaction mix (3 ml) through a 0.45 µm syringe filter into a suitable cuvette and record the $r_{A_{420nm}}$ using a suitably thermostatted spectrophotometer¹. This step should be repeated at least 4 times over seven minutes.

CALCULATIONS:

$$\text{Units/g agarose} = \frac{(r_{A_{420nm}/\text{min}})(30.1)(21)(1000)}{(2.64)(0.1)(15.7)}$$

30.1 = Total volume (in milliliters) of the assay

21 = Dilution factor

1000 = Conversion factor from mg to g

2.64 = Millimolar extinction coefficient² of purpurogallin at 420nm

0.1 = Volume (in milliliter) of enzyme used

15.7 = mg dry agarose/ml of suspension

UNIT DEFINITION:

One unit will form 1.0 µmole of purpurogallin from pyrogallol per minute at pH 6.0 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 30.1 ml reaction mix, the final concentrations are 11 mM potassium phosphate, 0.03% (w/w) hydrogen peroxide, 0.55% (w/v) pyrogallol, and 0.075 mg of dry agarose peroxidase, insoluble.

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REFERENCES:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume I, 473-474

NOTES:

1. Sigma currently uses a computer program written by Research Instruments International for use with UVIKON spectrophotometers which determines the amount of soluble and insoluble enzyme activity simultaneously.
2. The millimolar extinction coefficient was determined by Sigma.
3. This assay is based on the cited reference.
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.