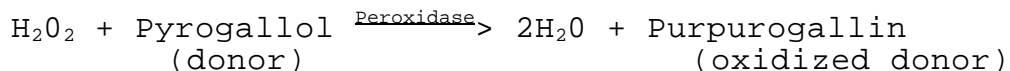


**Enzymatic Assay of PEROXIDASE
(EC 1.11.1.7)
from Soybean**

PRINCIPLE:



CONDITIONS: T = 20°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 20°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.0 at 20°C with 1.0 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% (w/v) Pyrogallol Solution
(Prepare 10 ml in deionized water using Pyrogallol, Sigma Prod. No. P-0381. **PREPARE FRESH AND KEEP FROM LIGHT.**)
- D. 0.1% (w/v) Bovine Serum Albumin (Enzyme Diluent)
(Prepare 50 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)
- E. Peroxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.4 - 0.7 unit/ml¹ of Peroxidase in cold Reagent D.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	2.10	2.10
Reagent A (Buffer)	0.32	0.32
Reagent B (H ₂ O ₂)	0.16	0.16
Reagent C (Pyrogallol)	0.32	0.32

Mix by inversion and equilibrate to 20°C. Monitor the A_{420nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Diluent)	-----	0.10
Reagent E (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in A_{420nm} for approximately 5 minutes. Obtain the r A_{420nm}/20 seconds using the maximum linear rate for both the Test and Blank.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{420\text{nm}}/20 \text{ sec Test} - \text{r } A_{420\text{nm}}/20 \text{ sec Blank})(3)(\text{df})}{(12)(0.1)}$$

sec = seconds

3 = Volume (in milliliters) of assay

df = Dilution factor

12 = Extinction coefficient of 1 mg/ml of Purpurogallin at 420 nm²

0.1 = Volume (in milliliters) of enzyme used

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

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

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UNIT DEFINITION:

One unit will form 1.0 milligram of 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.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 14 mM potassium phosphate, 0.027% (w/w) hydrogen peroxide, 0.5% (w/v) pyrogallol, 0.003% (w/v) bovine serum albumin, and 0.04 - 0.07 unit peroxidase.

REFERENCE:

Chance, B. and Maehly, A.C. (1955) *Methods in Enzymology*, II, 773-775.

NOTES:

1. The enzyme concentration may have to be modified in order for the rate, $\Delta A_{420\text{nm}}/20$ seconds, to be within the specified range of 0.7 - 0.9.
2. Extinction coefficient 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.