

Enzymatic Assay of PEROXIDASE¹ (EC 1.11.1.7)

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 A (Buffer)	-----	0.10
Reagent D (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in A_{420nm} for approximately 5 minutes. Obtain the $\Delta A_{420nm}/20$ seconds using the maximum linear rate² for both the Test and Blank.

CALCULATION:

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

s = seconds

3 = Volume (in milliliters) of assay

df = Dilution factor

12 = Extinction coefficient³ of 1 mg/ml of Purpurogallin at 420 nm

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 µM 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 and 0.04 - 0.07 unit peroxidase.

REFERENCE:

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

NOTES:

1. This assay procedure is not to be used to assay Peroxidase, Insoluble Enzyme attached to beaded agarose, Sigma Product No. P-3912, or Peroxidase, Sigma Prod. No. P-1432.
2. The enzyme concentration may have to be modified in order for the rate, $\Delta A_{420nm}/20$ seconds, to be within the specified range of 0.16 - 0.28.
3. Extinction coefficient determined by Sigma.
4. This assay is based on the cited reference.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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