ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of PEROXIDASE[1] (EC 1.11.1.7)

PRINCIPLE:

$$H_2O_2 + \text{Pyrogallol (donor)} \xrightarrow{\text{Peroxidase}} 2H_2O + \text{Purpurogallin (oxidized donor)}$$

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 (H_2O_2)
(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. Peroxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.4 - 0.7 unit/ml of Peroxidase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Deionized Water	2.10	2.10
Reagent A (Buffer)	0.32	0.32
Reagent B (H_2O_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 ΔA_{420nm}/20 seconds using the maximum linear rate[2] 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 coefficient[3] 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, ΔA_{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.