

Enzymatic Assay of GLYCEROL-3-PHOSPHATE OXIDASE¹ (EC 1.1.3.21)

PRINCIPLE:

DL-a-Glycerophosphate + O₂ $\xrightarrow{\text{GPO}}$ Dihydroxyacetone phosphate + H₂O₂

2H₂O₂ + 4-AAP + Phenol $\xrightarrow{\text{POD}}$ Quinoneimine Dye + 4H₂O

Abbreviations used:

GPO = Glycerol-3-Phosphate Oxidase

4-AAP = 4-Aminoantipyrine

POD = Peroxidase

CONDITIONS: T = 37°C, pH = 8.1, A_{500nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 125 mM Tris HCl Buffer with 0.125% (v/v) Triton² X-100, pH 8.1 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Triton² X-100 Sigma Stock No. X-100. Adjust to pH 8.1 at 37°C with 1 M HCl.)
- B. 200 mM DL-a-Glycerophosphate Solution (Substrate)
(Prepare 60 ml in Reagent A using DL-a-Glycerophosphate, Disodium Salt, Hexahydrate, Sigma Prod. No. G-2138. Readjust pH to 8.1 at 37°C with 1 M HCl or 1 N NaOH, if necessary.)
- C. 0.1% (w/v) 4-Aminoantipyrine Solution (4-AAP)
(Prepare 15 ml in deionized water using 4-Aminoantipyrine, Free Base, Sigma Prod. No. A-4382.)
- D. 0.1% (w/v) Phenol Solution (Phenol)
(Prepare 25 ml in deionized water using Phenol Sigma Prod. No. P-3653.)
- E. Peroxidase Enzyme Solution (POD)
(Immediately before use, prepare a solution containing 25 units/ml of Peroxidase from Horseradish, Sigma Prod. No. P-8250, in cold deionized water.)

Enzymatic Assay of GLYCEROL-3-PHOSPHATE OXIDASE¹
(EC 1.1.3.21)

REAGENTS: (continued)

- F. 0.25% (w/v) Sodium Dodecyl Sulfate Solution (SDS)
 (Prepare 25 ml in deionized water using Lauryl Sulfate, Sodium Salt, Sigma Prod. No. L-5750.)
- G. 20 mM Tris HCl Buffer with 0.2% (w/v) Bovine Serum Albumin, pH 7.5 at 37°C (Enzyme Diluent)
 (Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 at 37°C with 1 M HCl.)
- H. Glycerol-3-Phosphate Oxidase Enzyme Solution (GPO)
 (Immediately before use, prepare a solution containing 0.20 - 0.45 unit/ml of Glycerol-3-Phosphate Oxidase in cold Reagent G.)³

PROCEDURE:

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

Reagent B (Substrate)		25.00
Reagent C (4-AAP)	5.00	
Reagent D (Phenol)	10.00	
Reagent E (POD)		10.00

Mix by swirling and equilibrate to 37°C. Adjust the pH to 8.1 with either 1 M HCl or 1 M NaOH if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	1.00	1.00

Equilibrate to 37°C. Then add:

Reagent H (GPO)	0.02	-----
Reagent G (Enzyme Diluent)	-----	0.02

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

Reagent F (SDS)	2.00	2.00
-----------------	------	------

Enzymatic Assay of GLYCEROL-3-PHOSPHATE OXIDASE¹ (EC 1.1.3.21)

PROCEDURE: (continued)

Mix by swirling. Transfer the solutions to suitable cuvettes and record the $A_{500\text{nm}}$ for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{500\text{nm}} \text{ Test} - A_{500\text{nm}} \text{ Blank})(3.02)(\text{df})}{(10)(13.3)(0.5)(0.02)}$$

3.02 = Total volume (in milliliters) of stopped reaction

df = Dilution factor

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

13.3 = Millimolar extinction coefficient of Quinoneimine Dye at 500 nm under the assay conditions

0.5 = Conversion factor based on one mole of H_2O_2 produces half a mole of Quinoneimine Dye

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 oxidize 1.0 μmole of L-glycerol 3-phosphate to dihydroxyacetone phosphate with the formation of H_2O_2 per minute at 37°C at pH 8.1.

FINAL ASSAY CONCENTRATION:

In a 1.05 ml reaction mix, the final concentrations are 60 mM Tris, 0.06% (v/v) Triton, 0.01% (w/v) 4-aminoantipyrine, 0.02% (w/v) phenol, 5 units peroxidase, 95 mM DL- α -glycerophosphate, 0.01% (w/v) bovine serum albumin, and 0.004 - 0.009 unit glycerol-3-phosphate oxidase.

NOTES:

1. This assay is not to be used to assay Glycerol-3-Phosphate Oxidase, from *Streptococcus Thermophilus*, Sigma Prod. No. G-4388.

Enzymatic Assay of GLYCEROL-3-PHOSPHATE OXIDASE¹
(EC 1.1.3.21)

NOTES: (continued)

2. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co. Inc.
3. Initial dilution of enzyme should be to 1 mg/ml or higher.
4. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.
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.