

**Enzymatic Assay of GLYCEROL-3-PHOSPHATE OXIDASE  
(EC 1.1.3.21)  
from Streptococcus thermophilus**

**PRINCIPLE:**

DL-a-Glycerophosphate + O<sub>2</sub>  $\xrightarrow{\text{GPO}}$  Dihydroxyacetone Phosphate + H<sub>2</sub>O<sub>2</sub>

2H<sub>2</sub>O<sub>2</sub> + 4-AAP + Phenol  $\xrightarrow{\text{POD}}$  Quinoneimine Dye + H<sub>2</sub>O

Abbreviations used:

GPO = Glycerol-3-Phosphate Oxidase

4-AAP = 4-Aminoantipyrine

POD = Peroxidase

**CONDITIONS:** T = 37°C, pH 7.0, A<sub>505nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 500 mM Potassium Phosphate Buffer, pH 7.0 at 37°C  
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 37°C with 1 M NaOH.)
- B. 0.3% (w/v) 4-Aminoantipyrine Solution (4-AAP)  
(Prepare 10 ml in deionized water using 4-Aminoantipyrine, Free Base, Sigma Prod. No. A-4382.)
- C. 0.2% (w/v) Phenol Solution (Phenol)  
(Prepare 10 ml in deionized water using Phenol, Sigma Prod. No. P-3653.)
- D. 2 M DL-a-Glycerophosphate Solution (Substrate)  
(Prepare 50 ml in deionized water using DL-a-Glycerophosphate, Disodium Salt, Hexahydrate, Sigma Prod. No. G-2138.)
- E. Peroxidase Enzyme Solution (POD)  
(Immediately before use, prepare a solution containing 50 units/ml of Peroxidase, Sigma Prod. No. P-8250, in cold deionized water.)

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**REAGENTS:** (continued)

F. Glycerol-3-Phosphate Oxidase Enzyme Solution (GPO)  
(Immediately before use prepare a solution containing  
2 - 3 units/ml of Glycerol-3-Phosphate Oxidase in cold  
deionized water.)

**PROCEDURE:**

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

Reagent A (Buffer)	20.00
Reagent B (4-AAP)	10.00
Reagent C (Phenol)	10.00
Reagent D (Substrate)	50.00

Mix by swirling and adjust to pH 7.0 at 37°C with either  
1 M NaOH or 1 M HCl.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	0.90	0.90

Equilibrate to 37°C. Then add:

Reagent E (POD)	0.10	0.10
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Mix by swirling and equilibrate to 37°C. Monitor the  $A_{505nm}$   
until constant, using a suitably thermostatted  
spectrophotometer. Then add:

Reagent F (GPO)	0.01	-----
Deionized Water	-----	0.01

Immediately mix by inversion and record the increase in  
 $A_{505nm}$  for approximately 5 minutes. Obtain the  $r A_{505nm}/\text{minute}$   
using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{505nm}/\text{min Test} - r A_{505nm}/\text{min Blank})(1.01)(df)}{(13.3)(0.01)(0.5)}$$

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**CALCULATIONS:** (continued)

1.01 = Total volume (in milliliters) of assay  
df = Dilution factor  
13.3 = Millimolar extinction coefficient of  
Quinoneimine  
Dye at 505 nm under the assay conditions  
0.01 = Volume (in milliliter) of enzyme used  
0.5 = Conversion factor based on one mole of H<sub>2</sub>O<sub>2</sub>  
produces half a mole of Quinoneimine Dye

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

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

**UNIT DEFINITION:**

One unit will oxidize 1.0  $\mu$ mole of L-glycerol 3-phosphate to dihydroxyacetone phosphate with the formation of H<sub>2</sub>O<sub>2</sub> per minute at 37°C at pH 7.0.

**FINAL ASSAY CONCENTRATIONS:**

In a 1.01 ml reaction mix, the final concentrations are 99 mM potassium phosphate, 0.03% (w/v) 4-aminoantipyrene, 0.02% (w/v) phenol, 990 mM DL- $\alpha$ -glycerophosphate, 5 units peroxidase, and 0.02 - 0.03 unit glycerol-3-phosphate oxidase.

**REFERENCE:**

Esders, T.W. and Michrina, C.A. (1979) *Journal of Biological Chemistry* **254**, 2710-2715

**NOTES:**

1. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.