

**Enzymatic Assay of GLYCEROPHOSPHORYLCHOLINE PHOSPHODIESTERASE
(EC 3.1.4.2)**

PRINCIPLE:

L-a-Glycerophosphorylcholine + H₂O $\xrightarrow{\text{GPCP}}$ Glycerophosphate + Choline

Choline + O₂ $\xrightarrow{\text{Choline Oxidase}}$ Betaine Aldehyde + H₂O₂

Betaine Aldehyde $\xrightarrow{\text{Choline Oxidase}}$ Betaine + H₂O₂

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

Abbreviations used:

GPCP = Glycerophosphorylcholine Phosphodiesterase

4-AAP = 4-Aminoantipyrine

POD = Peroxidase

CONDITIONS: T = 37°C, pH = 8.0, A_{500nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 200 mM Tris HCl Buffer, pH 8.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl.)
- B. 10 mM L-a-Glycerophosphorylcholine Solution (GPC)
(Prepare 5 ml by pipetting 2.57 ml of L-a-Glycerophosphorylcholine, Sigma Prod. No. G-4007, into a 25 ml beaker. Evaporate the methanol under nitrogen gas and then reconstitute with 5 ml of Reagent A.)
- C. 10 mM Calcium Chloride Solution (CaCl₂)
(Prepare 10 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- D. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS.)

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PROCEDURE:

- E. 0.3% (w/v) 4-Aminoantipyrine Solution (4-AAP)
(Prepare 2 ml in deionized water using 4-Aminoantipyrine, Sigma Prod. No. A-4382.)
- F. 0.2% (w/v) Phenol Solution (Phenol)
(Prepare 2 ml in deionized water using Phenol, Sigma Prod. No. P-3653.)
- G. Choline Oxidase Enzyme Solution (Choline Ox)
(Immediately before use, prepare a solution containing 60 units/ml of Choline Oxidase, Sigma Prod. No. C-5896, in cold deionized water.)
- H. 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.)
- I. 10 mM Tris HCl Buffer, pH 8.0 at 37°C (Enz Dil)
(Prepare 10 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl.)
- J. Glycerophosphate Phosphodiesterase Enzyme Solution (GPCP)
(Immediately before use, prepare a solution containing approximately 0.30 unit/ml of Glycerophosphate Phosphodiesterase in cold Reagent I.)

PROCEDURE:

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

| | |
|------------------------|------|
| Reagent A (Buffer) | 1.00 |
| Reagent D (EDTA) | 2.00 |
| Reagent E (4-AAP) | 1.00 |
| Reagent F (Phenol) | 1.00 |
| Reagent G (Choline Ox) | 1.00 |
| Reagent H (POD) | 1.00 |
| Deionized Water | 3.00 |

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PROCEDURE: (continued)

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------------------|-------------|--------------|
| Reagent A (Buffer) | 0.10 | 0.10 |
| Reagent B (GPC) | 0.05 | 0.05 |
| Reagent C (CaCl ₂) | 0.05 | 0.05 |
| Deionized Water | 0.25 | 0.25 |

Equilibrate to 37°C. Then add:

| | | |
|---------------------|-------|-------|
| Reagent J (GPCP) | 0.05 | ----- |
| Reagent I (Enz Dil) | ----- | 0.05 |

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

| | | |
|-------------------|------|------|
| Reaction Cocktail | 1.00 | 1.00 |
|-------------------|------|------|

Mix by inversion and incubate at 37°C for 20 minutes. Then add:

| | | |
|-----------------|------|------|
| Deionized Water | 1.50 | 1.50 |
|-----------------|------|------|

Mix by inversion and record the A_{500nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{500\text{nm}} \text{ Test} - A_{500\text{nm}} \text{ Blank})(3)(\text{df})}{(10)(12)(0.5)(2)(0.05)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

12 = Millimolar extinction coefficient of quinoneimine dye at 500 nm

0.5 = Conversion factor derived from the fact that 2 μmoles of H₂O₂ produce 1 μmole of quinoneimine dye at 500 nm

2 = Conversion factor derived from the fact that 1 μmole of glycerophosphorylcholine produces 2 μmoles of H₂O₂

0.05 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will produce 1.0 μ mole of choline from L-a-glycerophosphorylcholine, G-4007, per minute at pH 8.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.50 ml reaction mixture, the final concentrations are 25 mM Tris, 1 mM glycerophosphorylcholine, 10 mM calcium chloride, and 0.015 unit glycerophosphorylcholine phosphodiesterase.

REFERENCES:

Hayaishi, O. (1955) *Methods in Enzymology*, Volume I 660-672

Keesey, J. (1987) in *Biochemica Information* (Keesey, J. ed) 1st ed, 19-20, Boehringer Mannheim Biochemicals, Indianapolis, IN

NOTES:

1. This assay is based on the cited references.
2. Choline Oxidase Unit Definition: One unit will form 1.0 μ mole of H₂O₂ from choline and H₂O per minute at pH 8.0 at 37°C.
3. 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.