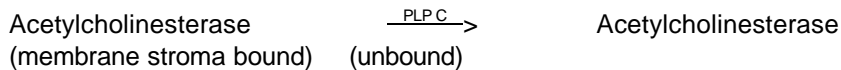


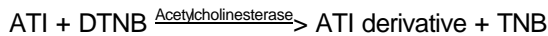
**Enzymatic Assay of PHOSPHOLIPASE C, PHOSPHATIDYLINOSITOL-SPECIFIC
(EC 3.1.4.10)**

PRINCIPLE:

Step 1:



Step 2:



Abbreviations:

PLP C = Phospholipase C, Phosphatidylinositol-Specific

ATI = Acetylthiocholine Iodide

DTNB = 5,5'-Dithio-bis(2-Nitrobenzoic Acid)

TNB = 5-Thio-2-Nitrobenzoic Acid

CONDITIONS: T = 30°C, pH = 7.4, A_{412nm}, Light path = 1 cm

METHOD: Stopped Spectrophotometric Rate Determination

REAGENTS:

- A. 10 mM Tris-HCl with 144 mM Sodium Chloride Buffer, pH 7.4 at 30°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 7.4 at 30°C with 1 M NaOH.)
- B. 200 mM Sodium Acetate Solution, pH 4.5 at 30°C (NaOAc)
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate Sigma Prod. No. S-8625. Adjust to pH 4.5 at 30°C with 1 M HCl.)
- C. 100 mM Sodium Phosphate with 0.1% (v/v) Triton¹ X-100 Solution, pH 7.4 at 30°C
(Phosphate/X-100)
(Prepare 50 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, and Triton¹ X-100, Sigma Stock No. X-100. Adjust to pH 7.4 at 30°C with 1 M NaOH.)

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REAGENTS: (continued)

- D. 1 mM Acetylthiocholine Iodide with
0.2 mM 5,5'-Dithio-bis(2-Nitrobenzoic Acid) Solution (ATI/DTNB)
(Prepare 50 ml in Reagent C using Acetylthiocholine Iodide, Sigma Prod. No. A-5751, and 5,5'-
Dithio-bis(2-Nitrobenzoic Acid), Sigma Prod. No. D-8130. Prepare this solution fresh every 4
hours since there is a spontaneous yellowing of the solution due to degeneration.)
- E. 0.001% (v/v) Phenylmethylsulfonyl Fluoride Solution (PMSF)
(Prepare 100 ml in Isopropanol, Anhydrous, Sigma Stock No. 405-7 using
Phenylmethylsulfonyl Fluoride, Sigma Prod. No. P-7626.)
- F. Acetylcholinesterase Membrane-Bound Enzyme Solution (AChE)
(Immediately before use, prepare a solution containing 6.25 mg/ml in Reagent A using
Cholinesterase, Acetyl, Sigma Prod. No. C-5021. Equilibrate to 37°C prior to use.)
- G. 0.05% (w/v) Bovine Serum Albumin Solution (Enz Dil)
(Prepare 20 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)
- H. Phospholipase C, Phosphatidylinositol-Specific Enzyme Solution (PLPC)
(Immediately before use, prepare a solution containing 0.05 - 0.10 unit/ml of Phospholipase C,
Phosphatidylinositol-Specific in Reagent G.)

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into suitable microcentrifuge tubes:

	<u>Test</u>	<u>Blank</u>
Reagent G (Enz Dil)	-----	0.10
Reagent H (PLPC)	0.10	-----

Equilibrate to 30°C. Then add:

Reagent F ² (AChE)	0.10	0.10
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PROCEDURE: (continued)

Mix by swirling and incubate at 30°C for exactly 10 minutes. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent B (NaOAc)	0.20	0.20

Mix by swirling and incubate at 4°C for 15 minutes. Centrifuge for 3 minutes. Place the supernatant from the Test and Blank into separate microcentrifuge tubes.

Step 2:

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

Reagent D (ATP/DTNB)	1.45	1.45
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Equilibrate to 30°C. Then add:

Test Supernatant	0.05	-----
Blank Supernatant	-----	0.05

Mix by swirling and incubate for exactly 10 minutes. Then add:

Reagent E (PMSF)	0.10	0.10
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Mix by swirling and transfer the Test and Blank to suitable cuvettes. Record the $A_{412\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{412\text{nm}} \text{ Test} - A_{412\text{nm}} \text{ Blank})(0.4)(1.6)(\text{df})}{(0.05)(10)(13.6)(0.1)}$$

1.6 = Total volume (in milliliters) of assay in Step 2

0.4 = Total Volume in (in milliliters) of assay in Step 1

df = Dilution factor

0.05 = Volume (in milliliter) of Step 1 used in Step 2

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

13.6 = Millimolar extinction coefficient³ of TNB at 412 nm

0.1 = 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 liberate 1.0 unit of acetylcholinesterase per minute from a membrane-bound crude preparation (C-5021) at pH 7.4 at 30°C (10 minute incubation).

FINAL ASSAY CONCENTRATION:

In a 0.20 ml reaction mix, the final concentrations are 10 mM Tris, 144 mM sodium chloride, 0.625 mg acetylcholinesterase, membrane-bound, 0.02% (w/v) bovine serum albumin, and 0.005 - 0.010 unit phospholipase C, phosphatidylinositol-specific.

REFERENCES:

Taguchi R., and Ikezawa H. (1987) *J. Biochem.* **102**, 803-811

Ellman, G.L. et al. (1961) *Biochem. Pharmacol.* **7**, 88-95

NOTES:

1. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.
2. Reagent F (AChE) should be equilibrated to 30°C before adding to the reaction mixture.
3. The millimolar extinction coefficient is described in Ellman, G.L. et al. (1961).
4. This assay is based on the cited references.
5. 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.