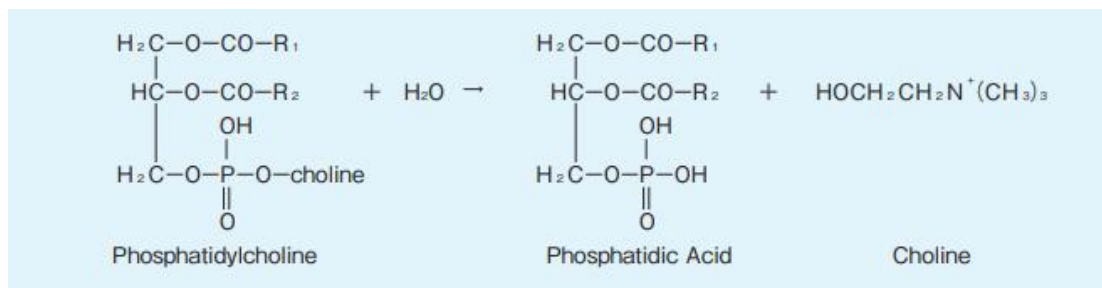


PHOSPHOLIPASE D



■ Principle

The assay is based on the increase in absorbance at 500 nm as the formation of quinoneimine dye proceeds in the following reactions:



COD: Choline oxidase

■ Unit definition

One unit is defined as the amount of enzyme which hydrolyzes 1 μmole of phosphatidylcholine to phosphatidic acid and choline per minute at 37°C under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture for the first reaction

0.2 M DMG–NaOH buffer pH 5.5 0.10 ml

10 mM CaCl₂ 0.05 ml

3% (W/V) Triton X–100 solution 0.05 ml

10 mM Substrate solution 0.15 ml

Distilled water 0.15 ml DMG: 3, 3'–Dimethylglutarate

1) : 10 mM Substrate solution Dissolve 78.6 mg of L–α–phosphatidylcholine, dioleoyl with 10 ml of 5% Triton X–100 (W/V) .

2. Reaction mixture for the second reaction



15 mM 4-AA	0.05 ml
0.2% (W/V) Phenol	0.05 ml
1 M Tris-HCl buffer, pH 8.0	0.05 ml
50 U/ml POD	0.05 ml
50 U/ml COD	0.05 ml
Distilled water	0.25 ml

2) : 50 U/ml POD Dissolve 500 U (PPU) of POD with 10 ml of distilled water.

3) : 50 U/ml COD Dissolve 500 U of COD with 10 ml of 10 mM Tris- HCl buffer, pH 8.0.

3. Reaction stopper

1 M Tris-HCl buffer containing 10 mM EDTA and 1% (W/V)
Cetyltrimethylammonium chloride EDTA: Ethylenediamine tetraacetic acid

4. Reaction dilution solution

1% (W/V) Triton X-100

5. Enzyme dilution buffer

10 mM DMG-NaOH buffer, pH 5.5 containing 0.1% Triton X-100

■ Enzyme solution

Weigh about 20 mg of test sample exactly and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette 0.45 ml of reaction mixture for the first reaction into a small test tube and preincubate them at 37°C.

2. After 5 min, add exactly 50 µl of enzyme solution and mix to start the first reaction.

※ In the case of a test blank, add 50 µl of enzyme dilution buffer in place of enzyme solution at this point.



3. After 10 min, add 0.50 ml of reaction stopper and mix. On stopping the first reaction, add 0.50 ml of the reaction mixture for the second reaction immediately to start the second reaction at 37°C.
4. After 20 min, add 1.5 ml of reaction dilution solution and mix.
5. After 10 min, measure the absorbance at 500 nm.

Absorbance

sample : As/min

blank : Ab/min

$$\Delta A/\text{min} = (A_s/\text{min} - A_b/\text{min}) \leq 0.40 \text{ Abs/min}$$

■ Calculation

$$\text{Activity (U/mg)} = \frac{\Delta A/10}{12.2 \times 1/2} \times \frac{1}{2} \times \frac{3.00}{0.05} \times \frac{1}{X}$$

12.2 : millimolar extinction coefficient of quinoneimine dye at 500 nm (cm² / μmole)

2 : the multiplier derived from the fact that 1 mole of phosphatidyl choline produces 2 mole of H₂O₂

1/2 : the multiplier derived from the fact that 2 mole of H₂O₂ produces 1 mole of quinoneimine dye

10 : reaction time (min)

3.00 : final volume (ml)

0.05 : volume of enzyme solution (ml)

X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20°C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition.

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