(90×210mm Size)

〈For Research Use Only〉 Code No. 296-63801 (1,300 Tests)

Wako LabAssay™ Phospholipid (Choline Oxidase・DAOS method)

〔Introduction〕

Phospholipids are a class of lipids, and a major component of all biological membranes, along with glycolipids, cholesterol and proteins.
LabAssay™ Phospholipid is based on an enzymatic methods using *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (DAOS) as a blue pigment. This kit is used for the quantitative determination of phospholipids in mouse serum. It is a simultaneous multi-sample assay format using a microplate, but measurements can also be made using a test tube.

〔Kit contents〕

(1)	Buffer (50mmol/L Good's buffer, pH7.5)	50mL	8vials
(2)	Chromogen Substrate (when reconstituted) Phospholipase D 0.47units/mL Choline Oxidase 2.0units/mL Peroxidase 4.2units/mL *N*-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (DAOS) 0.77mmol/L 4-Aminoantipyrine 0.24mmol/L Ascorbate Oxidase 3.9units/mL	For 50mL	8vials
(3)	Standard Solution Choline Chloride 54mg/dL (Corresponding to 300mg/dL Phospholipid)	10mL	2vials

〔Materials and apparatuses to be prepared〕

- 96-well microplate (transparent type)
- Micropipette
- Incubator maintaining at 37℃*
- Plate mixer*
- Microplate reader with a 600nm wavelength filter
  (* if the microplate reader is not equipped.)

(For test tube method)

- Test tube
- Pipette
- Spectrophotometer or colorimeter with 600 nm wavelength filter

〔Assay principle〕

Phospholipids (lecithin, sphingomyelin and lysolecithin) in a sample are hydrolyzed to choline in a reaction catalyzed by phospholipase D. Choline so formed is oxidized by choline oxidase in a reaction that produces hydrogen peroxide. The hydrogen peroxide produced causes DAOS and 4-Aminoantipyrine to undergo a quantitative oxidative condensation catalysed by peroxidase (POD), producing a blue pigment. The amount of

phospholipids contained in the sample is determined by measuring the absorbance of the blue color.

$$\text{Phospholipids} \begin{pmatrix}\text{Lecithin}\\ \text{Sphingomyelin}\\ \text{Lysolecithin}\end{pmatrix} + H_2O$$

$$\xrightarrow{\text{Phospholipase D}} \text{Choline} + \begin{pmatrix}\text{Phosphatidic Acid}\\ \textit{N}\text{-Acylsphingosyl Phosphate}\\ \text{Lysophosphatidic Acid}\end{pmatrix}$$

$$\text{Choline} + 2O_2 \xrightarrow{\text{Choline Oxidase}} \text{Betaine} + 2\,H_2O_2$$

$$\text{4-Aminoantipyrine} + \text{DAOS} + H_2O_2 \xrightarrow{\text{POD}} [\text{Blue Pigment}]OH^- + 3H_2O$$

〔Preparation of reagents to be used〕

① Color Reagent :
Prepare Color Reagent by Dissolving 1 vial of Chromogen substrate (for 50mL) to 50mL of Buffer. After reconstitution, the solution should be stored at 2-10℃ and used within one week.

② Standard solution (Microplate method)
Standard solution is prepared by dilution of the provided Standard.

No.	Phospholipid Standard	Distilled or deionized water	Sample volume	Phospholipid Concentration
1	100 μL	100 μL	2 μL	150 mg/dL
2	Undiluted	—	2 μL	300 mg/dL
3	Undiluted	—	4 μL	596.1 mg/dL*1

*1 The test sample volume is usually 2 μL, but 4 μL is taken in this case. The phospholipids concentration must be corrected accordingly as indicated in the table above.

〔Procedure〕

(1) Assay in a microplate
Perform the assay in the wells according to the following table scheme.

	Test	Standard	Blank
Sample	Serum 2 μL	Standard solution 2 μL	—
Color reagent	300 μL	300 μL	300 μL
	Mix well and incubate at 37℃ for 5 min. Measure the absorbance at 600nm*2 of the test sample and standard solution with the blank solution as the control.		

*2 In two wavelength assay, measure using Main wavelength 600nm/Sub wavelength 700nm.

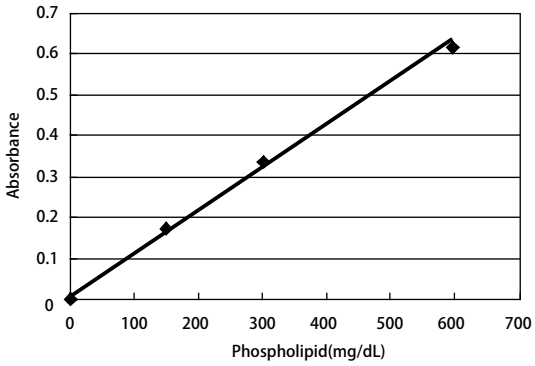
(2) Assay in a test tube

Perform the assay in a test tube according to the following table scheme.

	Test	Standard	Blank
Sample	Serum 0.02 mL	Standard solution 0.02 mL	—
Color reagent	3 mL	3 mL	3 mL
	Mix well and incubate at 37℃ for 5 min. Measure the absorbance of the test sample and standard solution with the blank solution as the control. Colorimeter with 600nm Spectrophotometer : 600nm*2		

*2 In two wavelength assay, measure using Main wavelength 600nm/Sub wavelength 700nm.

〔**Standard curve**〕〔assay in a microplate〕



Microplate reader : SAFIRE (TECAN)

〔**Performance**〕

(1) Sensitivity〔assay in a test tube〕

- The absorbance is below 0.14, when measuring purified water as a sample.
- The absorbance is 0.12 to 0.58, when measuring 300mg/dL phospholipid as a sample.

(2) Specificity

- The phospholipid concentration is less than ± 10%, when measuring the known concentration of control serum as a sample.

〔**Usage Notes**〕

(1) Sample

- Ascorbic acid, bilirubin and hemolysis may not significantly affect the assay.
- This method measures lecithin, sphingomyelin, lysolecithin but not cephalin.

(2) Notes on the assay

- Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures or for other purposes.
- Operate the measurement apparatuses according to operator's manuals under appropriate conditions. Consult the apparatus manufacturer for details.
- Keep the provided reagents under the indicated conditions before the expiration.
- The reaction completes in about 3 min. It is enough to incubate at 37 ℃ for 5 min. When the incubation is continued, the absorbance gradually decreases. Therefore, the incubation should be done within 15 min.
- Color level of the reaction solution should show very little change within 1 hour at room temperature.
- This kit is for research use only. Not for diagnostic use.

(3) Safety precautions

- If a reagent comes into contact with the mouth, eyes, or skin, immediately wash with a lot of water. Consult a physician if necessary.
- A pipette should be used with a safety pipette filler.

(4) Waste

- The waste should be processed appropriately according to the law.
- All the devices including reagents and vials that come into contact with specimens should be considered potentially infectious.

Expiration date : 12 months after the manufacture

Storage : Store at 2 ～ 10℃

Package : 1,300 tests

〔**Reference**〕

1. Takayama, M., Itoh, S., Nagasaki, T. and Tanimizu, I. : *Clin. Chim. Acta*., **79**, 93 (1977).

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