Phospholipids FS*

Diagnostic reagent for quantitative in-vitro determination of choline-containing phospholipids in serum and plasma on photometric systems

Order information

Cat. No.	Kit size
1 5741 99 10 930	R1 4 x 20 mL + R2 2 x 10 mL
1 5740 99 10 041	3 x 1 mL Standard

Summary [2,3]

Phospholipids are a major component of all biological lipid bilayer membranes. Phosphatidyl choline (known as lecithin), lysophosphatidyl choline and sphingomyelin make up 95% of the phospholipids in human blood plasma. Certain plasma phospholipid values seem to be affected by pathological conditions like liver disease, coronary heart disease and diabetes. Furthermore, some diseases are characterized by altered phospholipid concentrations or compositions in blood plasma, like obstructive jaundice, Tangier disease, hypobeta-lipoproteinemia or LCAT deficiency.

Method

Enzymatic colorimetric test

Principle

Phosphatidyl choline + H_2O $\xrightarrow{\text{Phospholipase D}}$ Choline + Phosphatidic acid

Choline + 2 O_2 + H_2O $\xrightarrow{\text{Choline oxidase}}$ Betaine + 2 H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + TBHBA $\xrightarrow{\text{Peroxidase}}$ Chinone dye + 4 H_2O

Reagents

Components and Concentrations

R1:	Tris buffer	pH 8.0	75 mmol/L
	TBHBA		3 mmol/L
	Choline oxidase		≥ 3 kU/L
R2:	Tris buffer	pH 8.0	75 mmol/L
	4-Aminoantipyrine		6 mmol/L
	Peroxidase		≥ 30 kU/L
	Phospholipase D		≥ 3.0 kU/L
Standard:			4 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents.

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes!
2. The reagents contain biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.
Avoid formation of foam. Do not shake the reagents.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum and plasma

Stability [4]

5 days	at	20 – 25°C
1 month	at	2 – 8°C
1 month	at	–20°C

Discard contaminated specimen! Only freeze once!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	570 nm
Optical path	1 cm
Temperature	37°C
Measurement	against reagent blank

	Blank	Sample/ standard
Sample/standard	-	10 µL
Dist. water	10 µL	-
Reagent 1	1200 µL	1200 µL
Mix and incubate for 5 minutes. Read absorbance A1, then add:		
Reagent 2	300 µL	300 µL
Mix and read absorbance A2 after exactly 5 minutes.		

ΔA = (A2 – A1) sample/standard

Calculation

With standard

$$\text{Phospholipids [mg/dL]} = \frac{\Delta A \ \text{Sample}}{\Delta A \ \text{Std.}} \times \text{Conc. Std. [mg/dL]}$$

Conversion factor

Phospholipids [mg/dL] x 0.0129 = Phospholipids [mmol/L]

Calibrators and Controls

For calibration, DiaSys TruCal Lipid or DiaSys Phospholipids Standard FS is recommended. The assigned values of the calibrator or standard are traceable to a primary standard material. DiaSys TruLab L should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal Lipid	1 3570 99 10 045	3 x 2 mL
TruLab L Level 1	5 9020 99 10 065	3 x 3 mL
TruLab L Level 2	5 9030 99 10 065	3 x 3 mL

* fluid stable

Performance characteristics

Measuring Range

The test has been developed to determine phospholipid concentrations within a measuring range from 0.09 – 13.3 mmol/L (7 – 1030 mg/dL). When values exceed this range, samples should be diluted 1+1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 0.09 mmol/L (7 mg/dL).

Precision

Intra-assay n = 20	Mean [mmol/L]	SD [mmol/L]	CV [%]
Sample 1	0.62	0.01	1.44
Sample 2	3.20	0.03	1.09
Sample 3	3.36	0.03	1.03

Inter-assay n = 20	Mean [mmol/L]	SD [mmol/L]	CV [%]
Sample 1	0.99	0.04	3.83
Sample 2	1.59	0.04	2.75
Sample 3	2.80	0.08	2.82

Method Comparison

A comparison of DiaSys Phospholipids FS (y) with a commercially available test (x) using 110 serum and plasma samples gave following results:
y = 0.830 x + 0.163 mmol/L; r= 0.996

Reference Range [1]

Serum/Plasma	mmol/L	mg/dL
Newborn:	0.90 – 2.19	70 – 170
Infant:	1.29 – 3.55	100 – 275
Child:	2.32 – 3.81	180 – 295
Adult:	1.61 – 3.55	125 – 275

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Pennell C, et al. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1788–1846.
2. Subbajah P.V. Determination and Clinical Significance of Phospholipids. In Rifai N. Warnick G.R Dominiczak M.H. Handbook of lipoprotein testing. 2 nd ed. AACC Press 2000. p. 521-36
3. Hilbert T, Lifshitz MS. Lipids and Dyslipoproteinemia. In: Clinical diagnosis and management by laboratory methods. 21st ed. Philadelphia. Saunders Elsevier 2007. p. 200–218
4. Data on file at DiaSys Diagnostic Systems GmbH.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45()): 1240–1243.

Manufacturer

IVD CE
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany