PHOSPHOLIPIDS

Trinder method - Endpoint

3 x 25 ml **REF CY05-75**

INTENDEDE USE

Kit for quantitative determination of Phospholipids in serum and in plasma according to Trinder reaction.

PRINCIPLE

Phospholipids are hydrolyzed by phospholipase into choline and acid residuals. Choline oxidase oxidizes choline into betaine, with formation of hydrogen peroxide, which in presence of peroxidase (POD), reacts with phenol and 4-aminophenazone, to form a colored complex whose intensity is directly proportional to phospholipids concentration in the sample.

SAMPLE

Serum, plasma. Avoid samples with high concentrations of ascorbic acid. Phospholipids in the sample are stable up to 3 days at 2-8°C and 3 months at -20°C.

REAGENTS

Only for in Vitro diagnostics.

Reagents	REF CY05-75	Quantity
REAGENT 1A (lyo) 4-aminophenazone 15 mmol/L, phospholipase 15 KU/L, choline oxidase 30 KU/L, POD 15 KU/L, excipient	CY05-75R1	3 vials
REAGENT 1B 4-aminophenazone 15 mmol/L, phospholipase 15 KU/L, choline oxidase 30 KU/L, POD 15 KU/L, excipient	CY05-75R2	3 x 25 ml
STANDARD (Std) Choline chloride corresponding to 300 mg/dl (3,88 mmol/L) of phospholipids, sodium azide 15 mmol/L	CY05-75S	1 x 4 ml

Stability: store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Do not freeze. Keep bottles closed when not in use. Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

PREPARATION OF WORKING REAGENT

Dissolve 1 vial of Reagent 1A with 1 vial of Reagent 1B.
Stability: 3 days at 15-25°C, 21 days at 2-8°C, if stored in a closed bottle protected from light.

MANUAL ASSAY PROCEDURE

Analysis:	increasing endpoint
Wavelength:	510 nm (500-520)
Cuvette:	1 cm optical path
Temperature:	37°C
Reaction Time:	10 minutes
Reading:	against blank reagent
Sample/Reagent:	1/125

Let the working reagent required to perform the test reach the chosen temperature for the analysis.

Pipette in cuvette:

	Blank Reagent	Standard	Sample
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl
Working reagent	1,25 ml	1,25 ml	1,25 ml

Mix. Incubate at 37°C for 10 minutes. Read the standard (AbsStd) and the sample absorbencies (AbsS) against the blank reagent.

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Calculate the phospholipids concentrations in the sample using the following formula to:

[mg/dl] phospholipids = AbsS / AbsStd x 300

[µmol/L] phospholipids = AbsS / AbsStd x 3,88

REFERENCE VALUES

Serum / plasma:

150 ÷250 mg/dl (1,96 ÷ 3,23 mmol/L)

Each laboratory should define its own reference values for this method.

QUALITY CONYTROL

A quality control program is recommended for all clinical laboratories.
Control sera in normal and high ranges for each assay are recommended.
The obtained values should be included within the manufacturer's accepted ranges for the method being used.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 5 mg/dl.

Linearity: up to 1000 mg/dl.
For higher values, dilute the samples with saline solution and multiply the result by 10.

Within-run precision:

	Level 1	Level 2
Average [mg/dl]	99,0	257,2
DS	2,6	5,6
CV %	2,7	2,2

Between-run precision:

	Level 1	Level 2
Average [mg/dl]	97,5	251,4
DS	2,3	7,8
CV %	2,3	3,1

Interferences: Bilirubin, hemoglobin and ascorbic acid in usual concentrations do not interfere with the test.

Correlation against a reference method: the correlation of the method (Y) against a reference method (X) gives this equation:

Y = 0,9865X + 2,2063 r = 0,9941

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

- The reagents may contain non-reactive components and various preservatives.
- Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behaviour in laboratory.
- Wear proper protective clothes - do not pipette by mouth
- read the kit instructions before performing the test
- use only the reagents contained in the kit and recommended reagents
- do not use reagents of different lots
- do not use reagents that have expired

REFERENCES

1. Takayama M., Itoh S., Nagasaki T., Tanimazu I. "Clin. Chem. Acta" 79,93 (1977).
2. Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. 1989.
3. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practise as specified in Council Directive 87/18/EEC.

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

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