Reactivos GPL

Barcelona, España

CE

- Phospholipids-

PHOSPHOLIPIDS

CHO-POD. Enzymatic-Colorimetric

Store at: +2+8ºC.

Presentation:
Cod. SU026 CONT: *R1 1 x 100 mL.+ R2 10 x 10 mL. CAL 1 x 5 mL.*

Procedure

Quantitative determination of phospholipids.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

Phospholipids are hydrolysed by phospholipase D and the liberated choline is subsequently oxidized by choline oxidase (CHO) to betaine with the simultaneous production of hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide couples oxidatively the 4 – Aminophenazone (4-AP) and dichlorophenol to forms a quinonemine dye:

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

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

$$2H_2O_2 + \text{4-AP} + \text{Dichlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + 4H_2O$$

The intensity of the red color formed is proportional to the glucose concentration in the sample[1,2].

REAGENTS COMPOSITION

R.1 (Buffer)	TRIS pH 7.55 Dichlorophenol	50 mM 2.1 mM
R.2 (Enzymes)	Phospholipase D Choline oxidase (CHO) Peroxidase (POD) 4 – Aminophenazone (4-AP)	400 U/L 2200 U/L 3600 U/L 1 mmol/L
Phospholipids Cal	Phospholipids aqueous primary standard	300mg/dL

REAGENT PREPARATION AND STABILITY

Working Reagent (WR): Dissolve (→) the contents of 1 vial R 2 Enzymes in 10 mL of R 1 Buffer. Cap and mix gently to dissolve contents.

The reagent is stable after reconstitution 3 weeks in the refrigerator (2-8ºC) or 7 days at 15-25ºC. Protect from the sunlight.

All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use.

Do not use reagents over the expiration date.

Phospholipids Cal: Proceed carefully with this product because due its nature it can get contaminated easily.

Signs of Reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm. ≥ 0.16

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-8ºC. Do not use reagents over the expiration date.

SPECIMEN

Serum or plasma.

Stability of the sample: 3 days at 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions
   - Wavelength : ..... 505 nm. (490-550).
   - Cuvette: ..... 1 cm light path.
   - Temperature .....37ºC.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a Cuvette:

	Blank	Calibrator	Sample
WR (mL.)	1.0	1.0	1.0
Calibrator(note1-2) (µL.)	--	10	--
Sample (µL.)	--	--	10

4. Mix and incubate for 5 min. at 37ºC.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\text{Phospholipids (mg/dL.)} = \frac{(A)\text{Sample}}{(A)\text{Calibrator}} \times 300 \text{ (Calibrator conc.)}$$

Conversion Factor. mg/dL. x 0.0129 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions

REFERENCE VALUES[1]

Adult: 125-275 mg/dL[1,6].

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Phospholipids are a complex lipid containing phosphorus.

Their function as the principal components of cell membranes makes phospholipids essential for all vital cell processes.The determination of serum phospholipids is an important clinical test in diagnosis of liver diseases, especially obstructive jaundice[1,2].

The serum phospholipids concentration in normal healthy individuals is in about the same concentration range as total cholesterol. The ratio of phospholipids to cholesterol remains 1/1. Any change in cholesterol concentration results in a corresponding change in phospholipids in similar direction.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE

– *Measuring Range*:
From detection limit of 2.54 mg/dL. to linearity limit of 600 mg/dL., under the described assay conditions.
If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.

– *Precision*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (g/dL)	121	221	126	225
SD	2.12	2.03	2.92	4.61
CV (%)	1.74	0.91	2.31	2.05

- *Sensitivity:*
1 mg/dL. = 0.0014A
- *Accuracy:*
Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCIES

– Interference:

No influence of ascorbic acid, glucose, bilirubin, uric acid or hemoglobin was found within the range of physiological concentration[2].

Other substances may interfere. A list of drugs and other substances that could interfere has been reported by Young et. al[3,4].

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

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