BIOLABO
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PHOSPHOLIPIDS

Colorimetric Enzymatic Method

Reagent for quantitative determination of Phospholipids in human serum or plasma

REF 99105	**R1** 10 x 50 mL	**R2** 10 x 50 mL	**R3** 1 x 10 mL
REF 99110	**R1** 10 x 100 mL	**R2** 10 x 100 mL	**R3** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (3)

Determination of phospholipids (PL) in serum is of great interest in the diagnosis of hepatic diseases, especially obstructive icteruses. In cardiovascular medicine, state of art allows to use phospholipids measurement to determine glycerides by subtraction of cholesterol and phospholipids from total lipids value. Elevated PL level is associated with various hyperlipidemias, cholestatic liver desease, Schistosomias infection and trauma. Decreased level of PL is associated with hematologic cancers, cerebrovascular desease.

PRINCIPLE (1) (2)

Enzymatic colorimetric method which reaction scheme is as follows:

Phospholipids $+H_2O$ —**Phospholipase D**→ Choline + phosphatidic acid + lysophosphatidic acid +N acylsphingosyl phosphate

Choline + $2O_2$ —**Choline Oxidase**→ Betaine + $2H_2O_2$

$2H_2O_2$ + Phenol + 4AAP —**POD**→ Quinoneimine + $4\ H_2O$

REAGENTS

Vial R1 **BUFFER**

Tris buffer	50	mmol/L
Chloro-4-phenol	3.5	mmol/L
$CaCl_2$	50	mg/L
Surfactant	1.5	mL/L
Stabilizer		

Vial R2 **ENZYMES**

Phospholipase D	≥ 450	UI/L
Peroxidase (POD)	≥ 3000	UI/L
Choline Oxidase	≥ 2000	UI/L
4 - Amino – antipyrine (4AAP)	0.25	mmol/L

Vial R3 **STANDARD**

Phospholipids	300	mg/dL
Preservative		

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advise.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Vial R2: If appropriate, use a non-sharp instrument to remove aluminium cap.

Add promptly the contents of vial R2 (Enzymes), into vial R1 (Buffer).

Mix gently until complete dissolution (approximately 2 minutes).

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8°C

- Standard (vial R3):transfer the requested quantity, recap and store at 2-8°C.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 2 weeks when free from contamination.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (3)

Serum (Heparin citrate, EDTA or oxalate, sodium fluoride) or plasma.

Phospholipids are stable in the specimen at least for:

- 7 days at 2-8°C

Freeze once only at -20° C for longer storage.

INTERFERENCES

Interferences studies have led to following results:

- Ascorbic acid (AA): Positive interference above 8 mg/dL
- Haemoglobin (Hb): No interference up to 434 µmol/L
- Glucose: No interference up to 1000 mg/dL
- Turbidity: No interference up to 0.311 abs at 600nm (milky serum)
- Bilirubin: No data yet available

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera

CALIBRATION

- Use vial R3 enclosed in the Kit
- Or any calibrator traceable to reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases:
1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- Any assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (4)

In serum or plasma:

Age	Phospholipids (mg/dL)
Newborn	75-170
Infant	100-275
Child	180-295
Adult	125-275
> 65 years	196-366

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES (PROCEDURE N°1)

Within run n = 20	*Low level*	*Medium level*	*High level*
Mean mg/dL	54	189	253
S.D. mg/dL	0.6	2	1
C.V. %	1	1.2	0.54

Between run n = 20	*Low level*	*Medium level*	*High level*
Mean mg/dL	52	130	252
S.D.mg/dL	2	4	5
C.V. %	3.7	3.1	1.9

Detection limit: approximately 10 mg/dL at 37°C.

Sensitivity for 100 mg/dL: approximately 149 mAbs at 37°C.

Comparison study with commercially available reagent: Not yet available

LINEARITY

Procedure n°1 (high sensitivity): linear up to 500 mg/dL.
Procedure n°2 (low sensitivity): linear up to 1000 mg/dL.

Above, dilute the specimen (1+4) with saline solution 9 g/L and reassay taking into account the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Procedure n° 1:

Pipette into well identified test tubes:	**Blank**	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL
Mix. Let stand for 10 minutes at 37°C Record absorbance at 500 nm (492-546) against reagent blank.			

Procedure n° 2:

Pipette into well identified test tubes:	**Blank**	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
Demineralised water	5 µL		
Standard		5 µL	
Specimen			5 µL
Mix. Let stand for 10 minutes at 37°C Record absorbance at 500 nm (492-546) against reagent blank.			

CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

REFERENCES

(1) *Subbaiah, P.V., Determination and clinical significance of phospholipids, p.521-p.531 in: Rifai N., Warnick G. R., Dominiczak M. H., Handbook of lipoprotein testing, 2nd éd., AACC Press 2000.*
(2) *Takayama Itoh S., Nagasaki T., Tanimizu I., Clin. Chem. Acta., 79, (1977), 93*
(3) *Tietz Clinical guide to laboratory test 4th Ed. (2006) p.850-853*
(4) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1829.*
(5) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995)*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with