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U Cinn - Phospholipids Assay

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Protocol status: Working
We use this protocol and it's working

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Abstract

Summary:

Determinations of phospholipids in plasma/serum/lymph will be made using the Wako Phospholipids C enzymatic assay. In this assay, phospholipids in the sample are hydrolyzed ultimately producing a blue pigment. The amount of phospholipids in the sample is determined by measuring the absorbance of the blue color.

Materials

MATERIALS

Phospholipid C assay kits standard included in kit **FUJIFILM Wako Pure Chemical Corporation Catalog #**433-36201

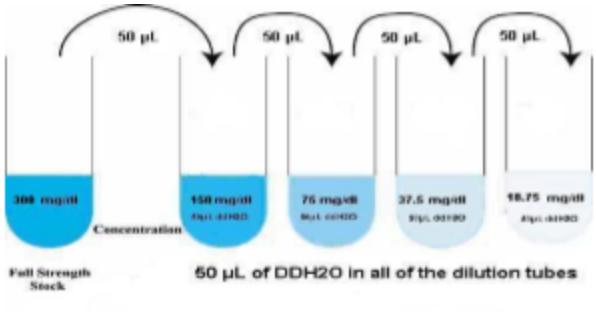
Working Reagent:
Reagents and Materials:
Color Reagent
Buffer

Procedure:
Reconstitute one vial of **Color Reagent** with a portion of **Buffer** then transferring entire contents to **Buffer** bottle, rinsing **Color** vial several times.

Note:
FUJIFILM Wako RRID:SCR_013651

Troubleshooting

1 Prepare working standards by making a serial dilution of the stock 300mg/dl standard. (See diagram below) *Stock standard included in kit.



2 Prepare **Working Reagent** by reconstituting one vial of **Color Reagent** with a portion of **Buffer** then transferring entire contents to **Buffer** bottle, rinsing **Color Reagent** vial several times.

3 Using a 96 well flat bottom plate, into separate wells, pipette 2µL of deionized water, standard, or sample to be assayed.

4 Add 300µL of **Working Reagent** to all wells.

5 Incubate plate for 5 minutes at 37°C.

6 Determine the absorbance (abs) of the standards and of each unknown at 600nm.

7 Calculate values of unknowns from the standard curve.

Specimen: Serum or Plasma. Specimen stable for 7 days at 2-8°C or 3 months at -20°C.
Assay Linearity: 1000 mg/dl
Reagent Stability: 7 days at 2-8°C
Stability of Final Reaction: 60 minutes