Aug 20, 2019

Ultracentrifugal separation of HDL alone and calculation of non-HDL

Daniel Teupser[1], Jan Breslow[1]

[1]Rockefeller University

1 Works for me dx.doi.org/10.17504/protocols.io.32mgqc6

Diabetic Complications Consortium
Tech. support email: rmcindoe@augusta.edu

Lili Liang

ABSTRACT

Summary:

This protocol is used to isolate the various lipid fractions from blood plasma using ultracentrifugation. The actual measured concentrations are performed separately once the isolations are complete.

NOTE: *This protocol IS applicable for ApoE knockout mice.*

Diabetic Complication:

Cardiovascular

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=17&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Beckman Optima TL tabletop ultracentrifuge		Beckman Coulter
Beckman 7x20 mm thick walled ultracentrifuge tube	343621	Beckman Coulter
Hamilton Syringe (100 ul)		
KBr Solution		
Phosphate Buffered Saline		

MATERIALS TEXT

Reagent/Material	Quantity Required
Beckman Optima TL tabletop ultracentrifuge	
Beckman 7x20 mm, thick walled ultracentrifuge tube	2
Hamilton Syringe (100 ul)	1
KBr Solution	1 ml
Phosphate Buffered Saline	1 ml

SAFETY WARNINGS

WARNING.

The use of an ultracentrifuge should only be performed by qualified technicians/personnel.

1 Add 60 ul of plasma to Beckman ultracentrifugation tube (7 x 20 mm; thick walled; polyallomer; cat. # 343621).

2 Layer 60 ul of PBS on top of the plasma and place tubes in a TLA100 rotor.

3 Spin for 3 hours Beckman Optima TL tabletop ultracentrifuge at 70,000 rpm, 4°C.

4 Using a 100 µl Hamilton syringe, carefully remove the bottom 60 µl and transfer to a new Beckman tube labeled with the sample number. Discard the upper portion of the sample (impure VLDL). Between samples rinse the Hamilton syringe with distilled water.

5 Add 60 µl KBr solution (density = 1.12 g/ml) to make a final density of 1.063 g/ml) and mix 5 to 6 times up and down with the same pipette tip.

6 Spin for 18 h overnight in the ultracentrifuge at 70,000 rpm at 4C as above.

7 Using a rinsed 100 µl Hamilton syringe remove the bottom 60 µl to a new Eppendorf tube labeled HDL. Discard the upper portion of the sample containing mostly LDL.

8 Measure cholesterol, triglycerides or phospholipids concentrations in the HDL fraction using their respective protocols.

9 The non-HDL is calculated by subtracting the HDL from the total.

The density of the HDL fraction is > 1.063 g/ml