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Ultracentrifugal separation of VLDL, LDL and HDL

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1 Works for me dx.doi.org/10.17504/protocols.io.33bgqin

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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 not applicable for ApoE knockout mice.

Diabetic Complications:

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=18&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 and A. Between samples rinse the Hamilton syringe with distilled water.

5 Using a rinsed Hamilton syringe transfer the rest of the sample (upper portion) into a second tube labeled with the sample number and B.

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

7 Layer 60 µl of PBS on top of the sample in tube B.

8 Spin both A and B for 18 h overnight in the ultracentrifuge at 70,000 rpm at 4C as above.

9 Using a rinsed 100 µl Hamilton syringe remove the bottom 60 µl from tube A and transfer to an Eppendorf tube labeled HDL. Using a rinsed Hamilton syringe transfer the remaining 60 µl (upper portion) to an Eppendorf tube labeled LDL.

10 Using a rinsed Hamilton syringe remove the bottom 60 µl from tube B and transfer to the same Eppendorf tube labeled LDL in step 9 above (To recover any LDL contaminating the VLDL preparation after the first ultracentrifugation spin).

11 Using a rinsed Hamilton syringe transfer the remaining 60 µl from tube B to an Eppendorf tube labeled VLDL.

12 Measure cholesterol, triglycerides or phospholipids concentrations in the lipoprotein fractions using their respective protocols.

> ***NOTE: When determining the lipid concentrations of the lipoprotein fractions, the value for LDL must be multiplied by 2 in order to account for the two-fold higher volume (120µl) in this tube.***

The densities of the fractions are as follows:
VLDL < 1.006 g/ml
LDL, IDL 1.006 – 1.063 g/ml
HDL > 1.063 g/ml