Ultracentrifugal separation of VLDL, LDL and HDL

Version: 1.0
Replaced by version: N/A
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NOTE: This protocol is not applicable for ApoE knockout mice.

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.

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Beckman Optima TL tabletop ultracentrifuge		Beckman	N/A
Beckman 7x20 mm, thick walled ultracentrifuge tube	2	Beckman	# 343621
Hamilton Syringe (100 ul)	1		
KBr Solution	1 ml	See Reagent Prep	
Phosphate Buffered Saline	1 ml	See Reagent Prep	

Protocol:

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.***

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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

Reagent Preparation:

KBr Solution
Phosphate Buffered Saline

KBr Solution:

Reagents and Materials

Reagent/Material	Quantity	Vendor	Stock Number

	Required		

Procedure

Phosphate Buffered Saline:

Reagents and Materials

Reagent/Material	Quantity Required	Vendor	Stock Number

Procedure