



Ultracentrifugal separation of HDL alone and calculation of non-HDL

Version: 1.0

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NOTE: This protocol IS 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 μ l of plasma to Beckman ultracentrifugation tube (7 x 20 mm; thick walled; polyallomer; cat. # 343621).
2. Layer 60 μ l 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 μ l Hamilton syringe, carefully remove the bottom 60 μ l 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 μ l 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 μ l Hamilton syringe remove the bottom 60 μ l 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

Reagent Preparation:

[KBr Solution](#)

[Phosphate Buffered Saline](#)

KBr Solution:

Reagents and Materials

Reagent/Material	Quantity Required	Vendor	Stock Number

Procedure

**Phosphate Buffered Saline:
Reagents and Materials**

Reagent/Material	Quantity Required	Vendor	Stock Number

Procedure