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

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 24365

Ultracentrifugal separation of HDL alone and calculation of non-HDL

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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 applicable for ApoE knockout mice.*

Diabetic Complication:

Cardiovascular

Keywords: Ultracentrifugal separation of HDL alone, calculation of non-HDL, cardiovascular

MATERIALS

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- Beckman Optima TL tabletop ultracentrifuge Beckman Coulter
- Beckman 7x20 mm thick walled ultracentrifuge tube Beckman Coulter Catalog #343621
- Hamilton Syringe (100 ul) Contributed by users
- KBr Solution Contributed by users
- Phosphate Buffered Saline Contributed by users

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 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