AUG 19, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.33bgqin

External link:
<https://www.diacomp.org/shared/document.aspx?id=18&docType=Protocol>

Protocol Citation: Daniel Teupser, Jan Breslow 2019. Ultracentrifugal separation of VLDL, LDL and HDL.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.33bgqin>

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

Created: Jun 11, 2019

Last Modified: Aug 19, 2019

PROTOCOL integer ID:
 24387

Ultracentrifugal separation of VLDL, LDL and 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 not applicable for ApoE knockout mice.

Diabetic Complications:

Cardiovascular

Retinopathy

Neuropathy

Nephropathy

Pediatric Endocrinology

Uropathy

Wound-Healing

Keywords:
ultracentrifugation, VLDL, LDL, HDL, cardiovascular, retinopathy, neuropathy, nephropathy, pediatric endocrinology, uropathy, wound-healing

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 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 μ l 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 μ l 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 μ l Hamilton syringe remove the bottom 60 μ l from tube A and transfer to an Eppendorf tube labeled HDL. Using a rinsed Hamilton syringe transfer the remaining 60 μ l (upper portion) to an Eppendorf tube labeled LDL.
- 10 Using a rinsed Hamilton syringe remove the bottom 60 μ l 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 μ l 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 (120 μ l) 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