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# Ultracentrifugal separation of HDL alone and calculation of non-HDL

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

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**Protocol Integer ID:** 24365

**Keywords:** Ultracentrifugal separation of HDL alone, calculation of non-HDL, cardiovascular, various lipid fractions from blood plasma, various lipid fraction, using ultracentrifugation, ultracentrifugation, hdl summary, lipid, hdl, blood plasma, diabetic complication

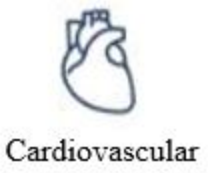
## Abstract

**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:**



## Materials

MATERIALS

- Beckman Optima TL tabletop ultracentrifuge **Beckman Coulter**
- Beckman 7×20 mm thick walled ultracentrifuge tube **Beckman Coulter Catalog #**343621
- Hamilton Syringe (100 ul)
- KBr Solution
- Phosphate Buffered Saline

| 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 |

## Troubleshooting

## 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 × 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. Discard the upper portion of the sample (impure VLDL). Between samples rinse the Hamilton syringe with distilled water.

5 Add 60 µl 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 µl Hamilton syringe remove the bottom 60 µl 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