

Rapid Separation of Human Serum Lipoproteins using the Thermo Scientific Sorvall Discovery M120 SE and M150 SE Microultracentrifuges

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KEY WORDS

- Lipoprotein Separation
- Microultracentrifuge
- S150-AT Rotor
- S140-AT Rotor
- S120-AT2 Rotor
- S120-AT3 Rotor

Introduction

Human serum lipoproteins are commonly isolated to study lipid metabolism and hyperlipidemia. The most frequently studied lipoproteins are VLDL (very low density lipoprotein), LDL (low density lipoprotein), and HDL (high density lipoprotein). VLDL particles ($\rho < 1.006$ g/mL) are formed when the liver synthesizes fats and packages them into a particle that is both hydrophobic and hydrophilic. This enables the VLDL and other lipoproteins to move freely in the bloodstream so that they may deliver lipids to various body cells. While in the bloodstream, lipoprotein lipase de-esterifies triglyceride in the VLDL creating a heavier particle. Some of these remnant particles are cleared by the liver, while the remainder is converted into an LDL particle (1.006 g/mL $< \rho < 1.063$ g/mL). The major transporter of cholesterol in humans is LDL. HDL apolipoproteins ($\rho > 1.063$ g/mL) are formed in both the liver and the intestine. The HDL particles retrieve cholesterol from various cells and transfer it to other lipoproteins for transport back to the liver for further metabolism or excretion.

Multiple rotors and protocols are available for use for the rapid separation of lipoproteins from human serum with the Thermo Scientific Sorvall Discovery SE microultracentrifuges (Table 1).

Procedures

The separation of serum lipoproteins occurs in a three-step process: 1) separation of VLDL, 2) separation of LDL, and 3) separation of HDL. An exception is the method described using the S120-AT3 rotor. The protocols are described below.

PROTOCOL 1: Using the S150-AT, S140-AT or S120-AT2 Rotor (See Figure 1 for schematic representation.)

1. Add Fat Red 7B to all samples for easier interpretation of results.
2. Layer 300 μ L of Solution A onto 600 μ L of serum.
3. Centrifuge at the appropriate time and speed indicated for your rotor choice listed in Table 1 for VLDL separation. (Acc. : 5; Dec. : 7)
4. Remove 300 μ L from sample 1 and mix remainder with 300 μ L of Solution B.



Thermo Scientific Sorvall Discovery SE Microultracentrifuge

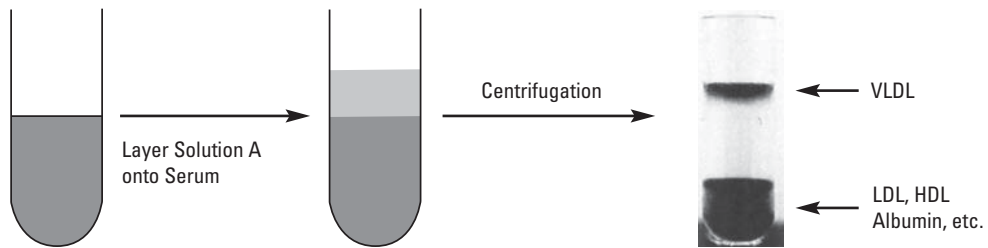
5. Centrifuge at the appropriate time and speed indicated for your rotor choice listed in Table 1 for LDL separation. (Acc. : 9; Dec. : 7)
6. Remove 300 μ L of sample 2 and mix remainder with 300 μ L of Solution C.
7. Centrifuge at the appropriate time and speed indicated for your rotor choice listed in Table 1 for HDL separation. (Acc. : 9; Dec. : 7)
8. All lipoprotein fractions can be stored at 4 $^{\circ}$ C.

Rotor	Speed (RPM)	G-Force (x g)	Temp ($^{\circ}$ C)	VLDL Separation Time (min.)	LDL Separation Time (min.)	HDL Separation Time (min.)
S150-AT	150,000	899,744	16	60	60	60
S140-AT	140,000	1,048,600	16	50	80	140
S120-AT2	120,000	649,826	8	85	125	210
S120-AT3*	120,000	649,826	10	90

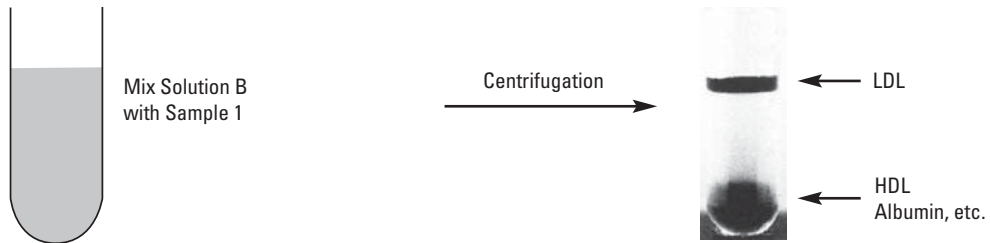
Table 1. Rotors and Protocols for Lipoprotein Separation.

*Due to the small volume of sample in this rotor, the separation protocol is a single-step process, 90 minutes in length, and will sediment LDL and HDL, or float LDL, depending on density.

(1) Separation of VLDL ($\rho < 1.006\text{g/cm}^3$)



(2) Separation of LDL ($1.006\text{g/cm}^3 < \rho < 1.063\text{g/cm}^3$)



(3) Separation of HDL ($1.063\text{g/cm}^3 < \rho < 1.21\text{g/cm}^3$)

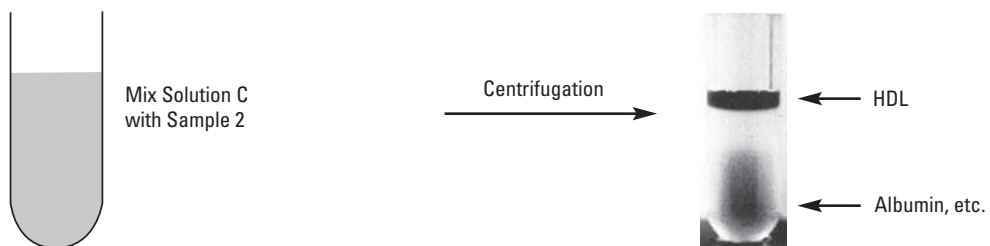


Figure 1. Separation of serum lipoprotein with the Thermo Scientific small volume fixed-angle rotors.

Solutions for Protocol 1

1. Solution A ($\rho: 1.006\text{ g/mL}$)

- Mix 11.40 g of NaCl and EDTA-2Na in a volumetric flask for 1,000 mL
- Add 500 mL of distilled water and 1 mL of 1M NaOH
- Mix until completely dissolved
- Add distilled water up to 1,000 mL
- Add an additional 3 mL of distilled water. (NaCl 0.195 mol)

2. Solution B ($\rho: 1.182\text{ g/mL}$)

Add 24.98 g of NaBr to 100 mL of Solution A. (NaCl: 0.195 mol, NaBr: 2.44 mol)

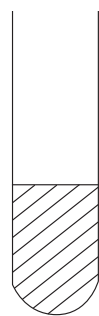
3. Solution C ($\rho: 1.478\text{ g/mL}$)

Add 78.32 g of NaBr to 100 mL of Solution A. (NaCl: 0.195 mol, NaBr: 7.65 mol)

PROTOCOL 2: Using the S120-AT3 Rotor (See Figure 2 for schematic representation.)

1. Add Fat Red 7B to all samples for easier interpretation of results.
2. Sedimentation of LDL and HDL
 - a. Mix 100 μ L of 0.15M NaCl, 0.3mM EDTA-Na₂ (pH 7.4, $\rho = 1.006$ g/mL) and 100 μ L of serum in a 0.5 mL polycarbonate thick-walled tube. (Catalog No. 45235)
 - b. Centrifuge at 120,000 rpm (649,826 x g) for 1.5 hr at 10 °C. (Acc. : 9; Dec. : 7)
3. Flotation of LDL
 - a. Mix 100 μ L of 15% (w/w) KBr ($\rho = 1.12$ g/mL) to 100 μ L serum in a 0.5 mL tube so that average density is 1.063 g/mL.
 - b. Centrifuge at 120,000 rpm (649,826 x g) for 1.5 hr at 10 °C. (Acc. : 9; Dec. : 7)

Sedimentation of LDL and HDL



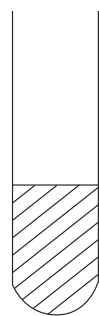
Sample : 200 μ l

Centrifugation



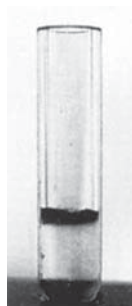
Lower layer :
HDL and LDL

Flotation of LDL



Sample : 200 μ l

Centrifugation



Top layer : LDL

Lower layer : HDL

Figure 2. Separation of lipoprotein from human serum using the Thermo Scientific S120-AT3 fixed-angle rotor.

Conclusion

Utilization of high g-forces sharply and efficiently separates small particles such as nucleic acids, lipoproteins, viruses and receptors. The separation of lipoproteins permits researchers and clinicians to study and evaluate levels of cholesterol, an indicator of cardiovascular health. Clinical laboratory testing of lipoprotein requires large numbers of samples and small sample volumes. The Thermo Scientific S150-AT, S140-AT, S120-AT2, and S120-AT3 fixed-angle rotors can help the research investigator save precious time by enabling clear, quick separation of lipoprotein.

References

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5. Separation of human serum lipoproteins at over 1 million x g with the S140-AT fixed angle Rotor. Application Brief, S00317.

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