

# himac APPLICATION

No. 81 June 1998

**Subject** Separation of serum lipoprotein using micro-ultracentrifuge

**Model** Preparative micro-ultracentrifuge, model: CS150GX

Separation of serum lipoprotein with S150AT fixed-angle rotor

Lipoprotein included in serum is closely related with metabolism of lipid. We have performed and introduce here separation of lipoprotein of VLDL, LDL, and HDL.

## 1. Instrument and implements

Instrument : Hitachi micro-ultracentrifuge, CS150GX

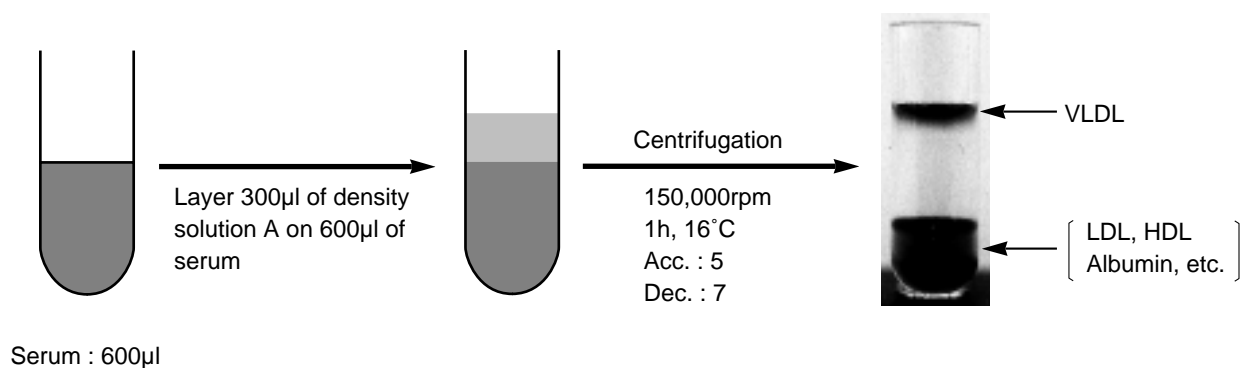
Rotor : S150AT fixed-angle rotor

Tube : 1PC tube

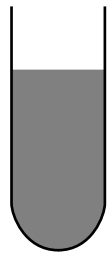
## 2. Procedure and results of separation

We added FAT RED 7B to the sample so that we could visually observe the experiments.<sup>1)</sup>

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

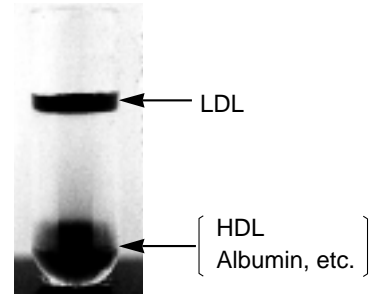


(2) Separation of LDL, including IDL ( $1.006\text{g/cm}^3 < \rho < 1.063\text{g/cm}^3$ )

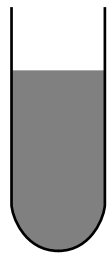


Mix well gently 300 $\mu$ l of density solution B and 600 $\mu$ l of sample after removing 300 $\mu$ l of (1).

Centrifugation  
150,000rpm  
1.5h, 16°C  
Acc. : 9  
Dec. : 7

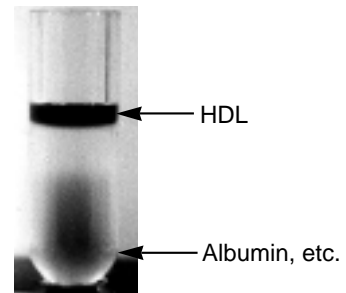


(3) Separation of HDL ( $1.063\text{g/cm}^3 < \rho < 1.21\text{g/cm}^3$ )



Mix well gently 300 $\mu$ l of density solution C and 600 $\mu$ l of sample after removing 300 $\mu$ l of (2).

Centrifugation  
150,000rpm  
2.5h, 16°C  
Acc. : 9  
Dec. : 7



Density solution A ( $1.006\text{g/cm}^3$ ) : Mix 11.40g of NaCl and EDTA-2Na in a volumetric flask for 1,000ml. Add 500ml of distilled water and 1ml of 1N NaOH, and stir them up so that they are dissolved completely. Measure up to 1,000ml and add 3 ml of distilled water (NaCl: 0.195mol).

Density solution B ( $1.182\text{g/cm}^3$ ) : Add 24.98g of NaBr to 100ml of density solution A (NaCl:0.195mol, NaBr: 2.44mol)

Density solution C ( $1.478\text{g/cm}^3$ ) : Add 78.32g of NaBr to 100ml of density solution A (NaCl:0.195mol, NaBr: 7.65mol)

(Reference)

1) T. J. Bronzert and H.B.Brewer, Clin. Chem., 23, 2089 (1977).

# HITACHI

**(Export office)**

Nissei Sangyo Co., Ltd.

Head Office :

1 - 24 - 14, Nishishinbashi, Minato-ku, Tokyo, 105 Japan

Tel : 81 - 3 - 3504 - 7281

Fax : 81 - 3 - 3504 - 7302

**(Manufacturer)**

Hitachi Koki Co., Ltd.

Scientific Instruments Div.

1060, Takeda, Hitachinaka - city, Ibaraki - pref., 312 Japan

Tel : 81 - 29 - 276 - 7384

Fax : 81 - 29 - 276 - 7475