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APPLICATION

January 2003

Separation of liver homogenate using the swinging bucket rotor for 5-ml tubes and the new-model micro ultracentrifuge

CS150GXL micro ultracentrifuge, S52ST swinging bucket rotor

Experiment: Separation of cell organelle from a rat liver homogenate using a swinging bucket rotor having 2.3 times higher capacity than the conventional rotor

Each cell organelle can be separated by the density gradient centrifugation with a homogenate of cells that constitutes organs such as liver. And, there is a possibility for getting informations such as diseases by examining the result of SDS-PAGE for the protein that originates in the cell organelle.

This time, a rat liver homogenate was separated using the density gradient solution Nycodenz[®] as a preliminary experiment.

Only the swinging bucket rotor for 2.2-ml tubes was usable for the micro ultracentrifuge until the new S52ST swinging bucket rotor that can contain 5-ml tubes came along. The new S52ST swinging bucket rotor has 2.3 times higher capacity than the conventional rotor. In addition, the new CS150GXL micro ultracentrifuge that is operable with the S52ST swinging bucket rotor is also added to our product line.

1. Equipment used

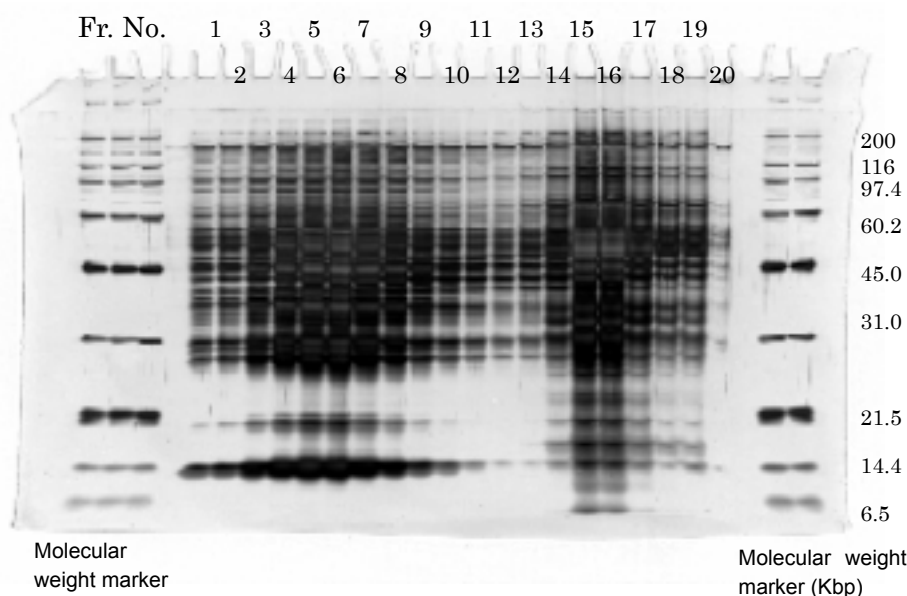
Centrifuge: Hitachi CS150GXL preparative micro ultracentrifuge

Rotor: S52ST swinging bucket rotor

(Max. speed 52,000 rpm, max. RCF 276,000 x g, capacity 5-ml tubes x 4 pcs.)

Tube: 5PA tubes

2. Result



Density range of Nycodenz[®]: 1.02 - 1.16g/ml (Initial concentration of Nycodenz[®]: 10%(w/v))

Fractionation quantity: 0.25 ml each for 20 fractionations

3. Separating conditions

Speed: 33,000 rpm
Max. RCF: 111,000xg (Average RCF: 79,500xg)
Time: 20 minutes
Temperature: 4°C
Acceleration mode: " 5 "
Deceleration mode: " 8 "
Amount of density gradient solution: 4.5 ml
Amount of sample: 0.4 ml
Concentration of sample: 10 mg/ml

4. Fractionation after centrifugation

Fractionator: Hitachi DGF-U fractionator
Fractionation: 0.25 ml x 20 fractions

5. Electrophoresis (SDS-PAGE) conditions

Concentration of gel: 10%
Amount of sample: 3 μ l

The above result was provided by Professor Kimie Murayama, Division of Biochemical Analysis, Central Laboratory of Medical Sciences, Juntendo University School of Medicine.

(References)

Kimie Murayama, Tsutomu Fujimura, Masataka Morita and Noriko Shindo, Electrophoresis, 2001, **22**, 2872-2880.

Nycodentz[®] is a trademark of Nycomed Pharma AS (Oslo, Norway).

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