Experiment: Separation of rat liver cell organelle using the continuous density gradient without use of density gradient former

Cell organelle can be separated from a homogenate of liver etc. by the density gradient centrifugation using the ultracentrifuge. However, there was a tendency to avoid density gradient centrifugation because the conventional methods required the use of a density gradient former and the operation was complicated.

This time, cell organelle were separated from a rat liver homogenate according to the procedure described in himac APPLICATION No. 108.

Continuous density gradient can be easily made according to the procedure below using Nycodenz® as the density gradient solution.

1. Equipment used
   - Centrifuge: CP-MX series preparative ultracentrifuge
   - Rotor: P28S swinging bucket rotor
   - Centrifugal tubes: 40PA tubes

2. Result of separation

![Fig. 1 Result of electrophoresis (1-D SDS-PAGE) for each fraction after centrifugation](Amount of sample: 3 μ l)
3. Separating conditions
   Speed: 25,000 rpm (Average RCF: 82,200xg)
   Time: 180 minutes
   Temperature: 4°C
   Sample: Rat liver homogenate
   Amount of sample: 3.5 ml (Concentration: About 14mg/ml)
   Density gradient solution: Nycodenz® (made by Nycomed Pharma AS (Oslo, Norway))
   Initial concentration of Nycodenz®: 20%(w/v) (1.105g/ml)
   Amount of Nycodenz®: 32 ml
   Amount of fraction: 0.75 ml/Fraction

A fractionator is required for fraction after centrifugation.  ALC-20 fractionator (made by Advantec Toyo Co., Ltd.) is reasonable and usable for fraction after centrifugation as well as Hitachi’s DGF-U fractionator.  Both fractionators are equipped with a level sensor and perform fractionation from the upper surface of the band in the centrifugal tube after centrifugation.

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

(References)

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