No.109

himac APPLICATION

January 2003

Easy preparation of density gradient using Nycodenz®

CP-MX series preparative centrifuge, swinging bucket rotor

Experiment: Easy preparation of 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 a density gradient former and the operation was complicated.
This time, we examined a new, easy method for density gradient centrifugation without any complication.

It is found that a continuous density gradient can be easily made according to the procedure below using Nycodenz® as the density gradient solution.

(1) Dilute Nycodenz® to the specified concentration.
(2) Freeze it at -20℃ or -80℃.
(3) Leave it at the ambient temperature to melt.

That is to say, you can obtain a continuous density gradient of Nycodenz® in the tube just by pouring the specified-concentration Nycodenz® in the tube, freezing it for preservation, taking it out of the freezer, and leaving it in the ambient temperature for melting.

1. Equipment used
   Medium of density gradient: Nycodenz® (made by Nycomed Pharma AS (Oslo, Norway))
   Centrifugal tubes: 5PA tubes or 40PA tubes
   Freezer (－20℃ or －80℃)
2. Initial concentration of Nycodenz®
   10％(w/v), 20％(w/v), 30％(w/v)
3. Result of density gradient

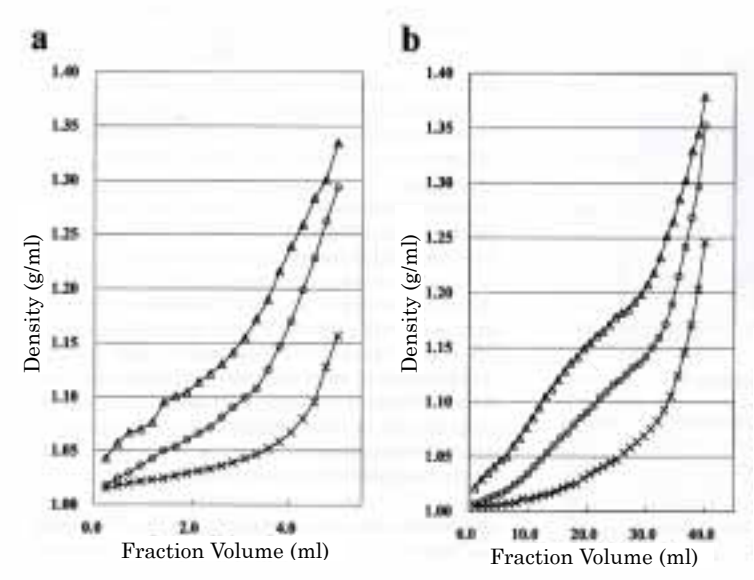

Fig.1
Effects of Nycodenz concentraion and tube size on the density gradient curves. (a)5PA tube (polyaromer,Hitachi 5mL), fractionation at 0.25mL/ tube. (b)40PA tube polyaromer, Hitachi 40mL),fractionation at 0.75mL/tube. Nycodenz con-centrations of (×)10%,(○)20%, and (△)30%

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

4. Range of prepared density gradient (at 20℃)

(1) 5PA tube (actual capacity: 4.9 ml) (Rotor:P65ST, P55ST2" or P50S2)

Initial concentration 10% (1.052g/ml): 1.015～1.157g/ml

Initial concentration 20% (1.105g/ml): 1.018～1.294g/ml

Initial concentration 30% (1.159g/ml): 1.043～1.334g/ml

(2) 40PA tube (actual capacity: 35.5 ml) (Rotor: P28S)

Initial concentration 10% (1.052g/ml): 1.005～1.246g/ml

Initial concentration 20% (1.105g/ml): 1.007～1.352g/ml

Initial concentration 30% (1.159g/ml): 1.021～1.379g/ml

5. Actual separation example

Centrifuge: CP-MX preparative micro ultracentrifuge

Rotor: P55ST2 swinging bucket rotor

Tube: 5PA tube

Speed: 28,000 rpm

Time: 20 minutes

Temperature: 4℃

Sample: Rat liver homogenate

Amount of sample: 0.4 ml (Concentration: 10mg/ml)

Initial concentration of Nycodenz®: 20%(w/v)

Amount of Nycodenz®: 4.5 ml

Amount of fraction: 0.25 ml/Fraction

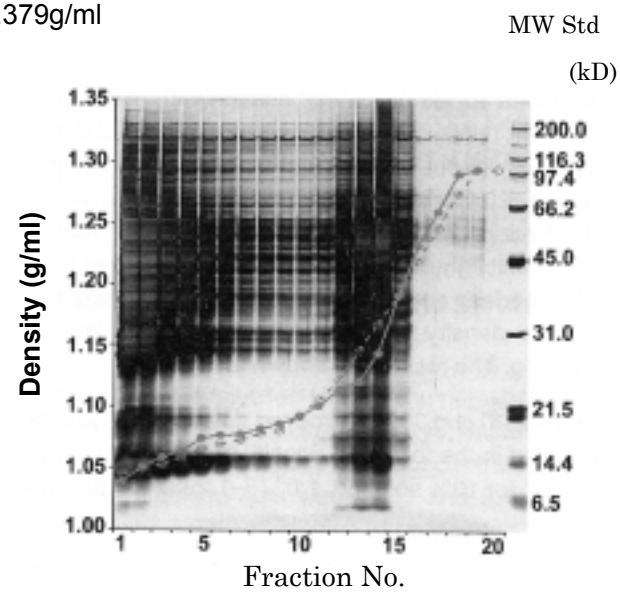

Fig.2

(Note)

This procedure is also applicable to the 13PA tube. However, in the case of using the 13PA tube, the density range 1.0444 - 1.2467g/ml (initial concentration 20%) is narrower than the other tubes and it is not useful (the 16PA tube is also the same). It is recommended to use the 5PA tubes or 40PA tubes for this procedure.

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.

HITACHI

Export offices
Hitachi High-Technologies Corporation
Head Office:
1-24-14, Nishishimbashi, Minato-ku, Tokyo, 105-8717 Japan
Tel: (81)3-3504-5461
Fax: (81)3-3504-7302

Manufacturer
Hitachi Koki Co., Ltd.
Scientific Instruments Division
1060, Takeda, Hitachinaka City, Ibaraki Pref., 312-8502 Japan
Tel: (81)29-276-7384
Fax: (81)29-276-7475

Nissei Sangyo (Singapore) Pte. Ltd.
Life Science Systems Dept.
10, Ang Mo Kio Street 65, #05-15 Techpoint, Singapore 569059
Tel: (65)481-2050
Fax: (65)481-8089

For the most current information, please access
http://www.hitachi-koki.co.jp/himac/