

himac

APPLICATION

January 2003

Separation of cell organelle by means of a new-model fixed angle rotor

CS-GXL series preparative micro ultracentrifuge, S55A fixed angle rotor

Examination: Separation of cell organelle by means of a rotor (13.5-ml tubes and 35° of cavity angle) designed for micro ultracentrifuges

Separation of cell organelle from a homogenate of liver etc. by means of a centrifuge is called "cell fractionation". In most cases, initial rough separation for the cell fractionation is done by differential pelleting without the use of density gradient solutions. A fixed angle rotor is used for the initial separation because it is suitable for pelleting. The new S55A fixed angle rotor has the same capacity as the commonest fixed angle rotors (popularly called "rotors intended for 12-ml tubes") designed for preparative ultracentrifuges, and its cavity angle is 35°. It is larger than the conventional rotors' cavity angle (24° – 26°). This time, separation of cell organelle from a rat liver homogenate was examined using the new S55A fixed angle rotor as described below.

1. Equipment and sample used

Centrifuge: CS150GXL micro ultracentrifuge
Rotor: S55A angle rotor
Centrifugal tubes: 10PC bottles
Sample: Rat liver homogenate
Amount of sample: 8 ml (per bottle)

2. Centrifugal conditions

①First centrifugation (Rough mitochondria fraction)

Speed: 12,000 rpm (Average RCF: 9,400xg)
Time: 20 minutes
Temperature: 4 °C
ACCEL and DECEL modes: "9" (Maximum acceleration) and "9" (Maximum deceleration)

②Second centrifugation (Rough microsome fraction)

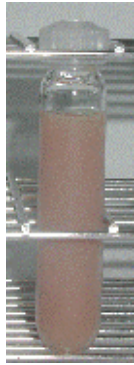
Speed: 41,000 rpm (Average RCF: 109,600xg)
Time: 1 hour
Temperature: 4 °C
ACCEL and DECEL modes: "9" (Maximum acceleration) and "9" (Maximum deceleration)

3. Specifications of S55A angle rotor

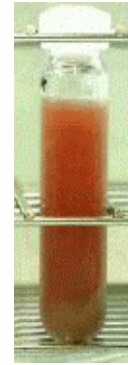
Maximum speed: 55,000 rpm
Maximum RCF: 260,000xg
Rotor's cavity angle: 35°
Rotor capacity: 13.5 ml tube × 8 pcs. = 108 ml
Actual capacity when using 10PC bottles: 8.5 ml tube × 8 pcs. = 68 ml

4. Result of separation

① First centrifugation



12,000 rpm, 20 minutes, 4°C



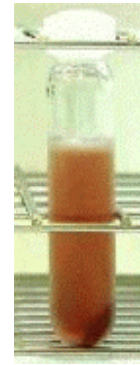
Before centrifugation

Precipitation: Rough mitochondria fraction

② Second centrifugation (Supernatant of the above ① is used.)



41,000 rpm, 1 hour, 4°C



Supernatant after the first centrifugation

Precipitation: Rough microsome fraction

Supernatant : Cytosolic protein fraction

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/>