

Separation of mitochondria by swinging bucket rotor designed for using conical bottom centrifuge tubes in high-speed refrigerated centrifuges

CR-G III series high-speed refrigerated centrifuges / R8S swinging bucket rotor

Mitochondria are widely known as an organelle inside cells of higher organisms for energy conversion and separation of mitochondria has been done for a long time since Dr. Albert Claude who won the Nobel Prize in Physiology or Medicine in 1974 established the centrifugation method of mitochondria. Mitochondria are separated by high-speed centrifugation and a swinging bucket rotor used for centrifugation at high RCF over 5,000 x g must be covered with a shell because of its structure. However, the shell causes some problems such as slow acceleration or deceleration and poor cooling capacity.

Following is an example on separation of rat liver mitochondria by means of the new, uniquely shaped R8S swinging bucket rotor that can be used for high-speed refrigerated centrifugation up to 11,500 x g without shell and hold commercially available 50ml conical bottom centrifuge tubes (TC tubes).

Experiment

1. Instruments

Centrifuge: CR-G III series high-speed refrigerated centrifuge
Rotor: R8S swinging bucket rotor (Up to 4 tubes can be contained.)
Tube: 50ml conical bottom centrifuge tubes (Commercially available)

2. Separation procedure

10% rat liver homogenate (Amount of tissue: 10 to 20 g)

↓ Perform centrifugation. (600 x g (1,800 rpm), 10 minutes, 4°C, Acc. 9, Dec. 9)

↓ Pour the supernatant into the new 50TC conical tube.

↓ Perform centrifugation. (5,500 x g (5,600 rpm), 20 minutes, 4°C, Acc. 9, Dec. 9)

↓ Remove the supernatant. When decanting, lightly shake the tube a couple of times to flush out the red-brown lysosomal fraction at the upper part of the sediment. Add appropriate amount of 0.25M sucrose solution to the yellow-brown sediment of the remained mitochondria and suspend again.

↓ Perform centrifugation. (6,000 x g (5,800 rpm), 15 minutes, 4°C, Acc. 9, Dec. 9)

↓ The more the above operation is repeated, the more the purity is increased. However, the activity is decreased as the purity is increased. So it is recommended to repeat the above operation twice only.

↓ Perform centrifugation. (6,000 x g (5,800 rpm), 15 minutes, 4°C, Acc. 9, Dec. 9)

↓ Sediment: Mitochondria fraction

The amount of mitochondria obtained by the above method is equivalent to 15 to 30 mg of protein content per gram of rat liver.

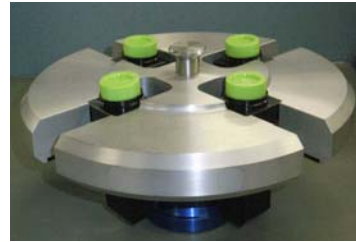
Reference: UP biology "Mitochondria" (Published by University of Tokyo Press in 1978)

Instrument

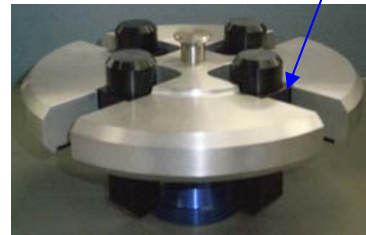
Operable at 5,000 x g or higher RCF without shell!



CR22G III high-speed refrigerated centrifuge



Shield bucket
(Sealing Cap: Option for Biosafety)



R8S swinging bucket rotor

If you have any inquiry of this application or products, please contact us through our web site.

<http://www.hitachi-koki.com/himac.contact/index.htm>

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