No.123

himac APPLICATION

Jun. 2004

Reliable separation of cell membranes by means of 50-ml conical tube

R18A angle rotor for high-speed refrigerated centrifuge

Separation of rat liver cell membranes by means of the 50-ml conical tube (***himac 50TC tube***) having tolerance for high RCF

1. Separation protocol

A liver of a fully grown rat

Homogenate

Perform centrifugation at 4,000 rpm (2,000xg), 4℃ for 15 minutes using the R18A angle rotor.

Pellets — Supernatant

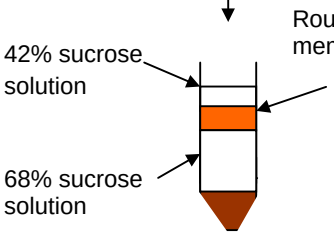
Suspend in 1mM $NaHCO_3$.

Perform centrifugation at 4,000 rpm (2,000xg), 4℃ for 15 minutes using the R18A angle rotor.

Pellets — Supernatant

1) Suspend in 1mM NaHCO3 at 1 g/ml of initial weight in the wet.
2) Suspend in a 68% sucrose solution.
3) Overlay a 42% sucrose solution.

Perform centrifugation at 18,000 rpm (42,200xg), 4℃ for 90 minutes using the R18A angle rotor. (ACCEL mode "5", DECEL mode "5")



Rough cell membranes

Suspend in 1mM $NaHCO_3$.

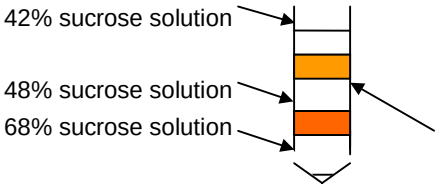
Perform centrifugation at 4,000 rpm (2,000xg), 4℃ for 30 minutes using the R18A angle rotor.

Repeat this operation twice.

Pellets — Supernatant

1) After suspension in 1mM $NaHCO_3$;
2) Suspend in 68%sucrose solution.
3) Overlay a 48% sucrose solution.
4) Overlay a 42% sucrose solution.

Perform centrifugation at 18,000 rpm (42,200xg), 4℃ for 90 minutes using the R18A angle rotor. (ACCEL mode "5", DECEL mode "5")



2. Explanation

The 50-ml conical tube is very popular in the field of biotechnology. This tube is reasonable and factory-sterilized by electron or γ-rays normally. In addition, it is an airtight tube. It is widely used for cultivation, preparation of homogenates, cell disintegration using surfactants or protease enzymes, phenol-chloroform extraction, and low- or middle-speed centrifugation. However, it cannot bear high RCF and the use is limited to operations up to the speed 12,000 rpm and the RCF 18,800xg (R12A5 fixed angle rotor). Thus, the 50-ml conical tube is mainly used for preparation works such as cell disintegration. The ultracentrifuge tubes that can bear high RCF are generally used instead of the 50-ml conical tubes for samples such as cell membranes requiring tens of thousands of RCF (xg) for purification.

This time, we have developed a new 50-ml conical tube (himac 50TC tube) that can be used at the maximum speed 18,000 rpm and the maximum RCF 42,200 x g, and the fixed angle rotor specifically designed for this tube. The himac 50TC tube is less expensive and more convenient than the conventional ultracentrifuge tubes. We have experimented that the new tube and rotor are applicable to separation of rat liver cell membranes.

A protocol of separating cell membranes from the homogenate of a fully-grown rat liver is shown in the previous page. As shown in this protocol, it is expected that the himac 50TC tube can be used in all the processes from the initial centrifugation of the homogenate sample to the final centrifugation using sucrose step density gradients for separation of purified cell membranes. It is also expected that the himac 50TC tube can be used for separation of mitochondria, lysosomes, peroxisomes, etc.

Refer to the following reference literature for details on handling samples etc.

(Reference literature)
New basic biochemistry experimental technique “Handling biological materials”
(Maruzen Co., Ltd.) (1987)

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