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himac APPLICATION

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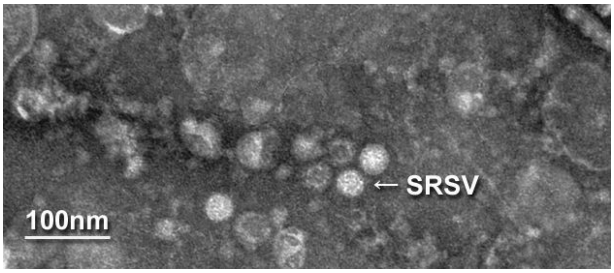
Separation of small range structured virus (SRSV) by means of a micro ultracentrifuge

CS-GX series micro ultracentrifuges and S80AT3 fixed angle rotor

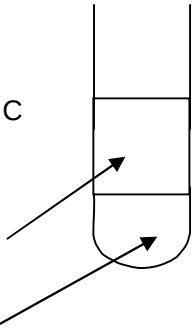
Example of separating virus in half the time of a conventional rotor (1.5 hours)

A large ultracentrifuge and a swinging bucket rotor have been used for separation of relatively small virus such as small range structured virus (SRSV), and it takes **4 hours or more** in general. We carried out an experiment on quick separation of SRSV by means of S80AT3 fixed angle rotor that was developed especially for differential centrifugation of virus and cell organelles. As a result, S80AT3 fixed angle rotor separated SRSV in **1.5 hours**. It reduces the time required for separation by more than half the conventional rotor.

Result of separation (Electron microscope photo: Hitachi H-7000)



Centrifuge: CS150GX micro ultracentrifuge
Rotor: S80AT3 fixed angle rotor
Tube: 6PC thick-walled tube
Speed: 45,000 rpm
Time: 1.5 hours
Temperature: 4 degrees C
Acceleration mode: 5
Deceleration mode: 7
Amount of sample: 3 ml
Cushioning solution
(30% (w/w) sucrose): 2 ml



Operating procedure

1. Pretreatment of sample
   1) Put about 1 g of patient's feces in a 15-ml polypropylene tube.
   2) Add 9 ml of distilled water to make a 10% suspension.
   3) Perform centrifugation under the following set conditions: 4,000 x g (with the angle rotor for high-speed micro centrifuges: 6,000 to 7,000 rpm), 30 minutes, 4 degrees C
   4) Put the supernatant in a new tube. Add equivalent-amount fluorocarbon (HCFC141b) and keep stirring for 2 minutes.

5) Perform centrifugation under the following set conditions: 2,000 x g (with the angle rotor for high-speed micro centrifuges: 3,500 to 4,000 rpm), 30 minutes, 4 degrees C.

6) Use the supernatant as a sample for centrifugation with the ultracentrifuge (Preservable by freezing).

2. Conditions of centrifugation with the ultracentrifuge

See the description at the right of the electron microscope photo in previous page.

3. Procedures after centrifugation with the ultracentrifuge

1) After centrifugation, take out the tube from the rotor and remove the brown supernatant and the 30% sucrose solution with a pipette.

2) Make a twisted string of KIMEWIPES and absorb the liquid adhered to the internal surface of the tube with the string. (At this time, be careful not to touch the precipitation.)

3) Add 30 μl of pure water. Cover the top of the tube with Parafilm.

4) Incline the tube so that the pure water contacts the precipitation and let it stand overnight at 4 degrees C.

5) Shake the tube for about 30 minutes with shaking equipment.

6) Put all the contents of the tube in a 1.5-ml micro tube.

7) Perform centrifugation at 15,000 rpm, 4 degrees C for 30 minutes.

8) Take out 1 to 2 micro L of the supernatant and perform negative coloring to examine it under a transmission-type electron microscope.

The above information including the electron microscope photo was provided by Dr. Etsuko Utagawa, National Institute of Infectious Disease, Department of Virology II.

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