No.104

himac APPLICATION

October 2000

Separation of SRSV with a swinging bucket rotor (5 ml x 4 tubes) in the new micro ultracentrifuge

CS150GXL micro ultracentrifuge and S52ST swinging bucket rotor

Example: Separation of SRSV (small range structured virus) with a swinging bucket rotor that has 2.3 times higher capacity than the conventional rotor

Conventional practices require a large-scale ultracentrifuge and its applicable swinging bucket rotor for separating relatively small spherical viruses such as SRSV. The new S52ST swinging bucket rotor for micro ultracentrifuge was developed. The S52ST swinging bucket rotor is capable of using 5 ml tubes that were used for large-scale ultracentrifuges only. In addition, the new CS150GXL micro ultracentrifuge that can use the new S52ST swinging bucket rotor was also developed. This time, we provide the centrifugal conditions for inspection of SRSV included in human feces with an electron microscope. A swinging bucket rotor is most desirable for such separation using gradient solution (cushion solution). The centrifugal conditions stated herein are also applicable to other viruses.

1. Equipment used

   Centrifuge: Hitachi CS150GXL preparative micro ultracentrifuge

   Rotor: S52ST swinging bucket rotor

   (Max. speed: 52,000 rpm, max. RCF: 276,000 x g, max. capacity: 5 ml x 4 tubes)

   Tube: 5PA tubes

2. Centrifugal conditions

   Speed: 38,000 rpm

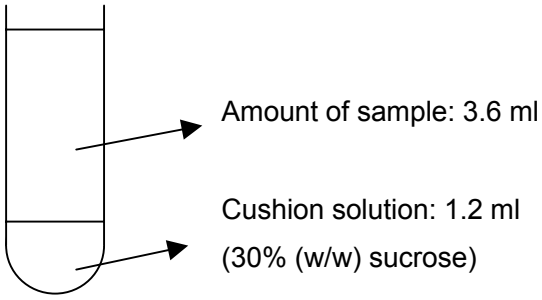
   Max. RCF: 147,000 x g (Average RCF: 105,000 x g)

   Time: 3 hours

   Temperature: 4 degrees centigrade

   Acceleration: “5”

   Deceleration: “7”



3. Procedures before centrifugation with an ultracentrifuge

(1) Put about 1 g of feces of the patient in a 15 ml centrifugal tube made of polypropylene.

(2) Add 9 ml of distilled water to make a 10% suspension.

(3) Perform centrifugation using a fixed angle rotor for high-speed micro centrifuge at 6,000 to 7,000 rpm (at about 4,000 x g) 4 degrees centigrade for 30 minutes.

(4) Remove the supernatant to a new centrifugal tube and add the same amount of fluorocarbon (HCFC141b). Stir it for 2 minutes.

(5) Perform centrifugation using a fixed angle rotor for high-speed micro centrifuge at 3,500 to 4,000 rpm (at about 2,000 x g) 4 degrees centigrade for 30 minutes.

(6) The supernatant is used as a sample for centrifugation with an ultracentrifuge (freezing available).

4. Procedures after centrifugation with an ultracentrifuge

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

(2) Make a twisted string of KIMEWIPER® and absorb the fluid deposited on the internal surface of the tube with the string. (At this time, be careful not to touch the sediment.)

(3) Add 36 micro litters of pure water.

(4) Check that the pure water covers the surface of the sediment. Cover the top of the centrifugal tube with PARAFILM® and let it stand overnight at 4 degrees centigrade.

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

(6) Remove all the contents of the centrifugal tube to a 1.5 ml micro tube.

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

(8) Remove 1 to 2 micro litters of the supernatant and perform negative coloring to examine it under a transmission-type electron microscope.

The above procedures were prepared according to the protocol provided by Dr. Etsuko Utagawa, National Infectious Disease Institute, Virus Dept. II.

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