himac APPLICATION
No.140
Dec. 2008

Separation of influenza viruses using fixed angle rotor designed for tabletop micro ultracentrifuge

CS150NX tabletop micro ultracentrifuge / S50A fixed angle rotor

Influenza viruses are classified in Orthomyxoviridae and the genome is single-strand RNA. There are three kinds of influenza viruses depending on serotype, type A, type B and type C. They are almost spherical viruses that are between 80 and 120 nm in diameter. In recent years in particular, highly-virulent avian flu viruses have been raising serious concerns about flu pandemic.

Following is an example on separation of influenza viruses by means of the S50A fixed angle rotor that is developed for the new table-top ultracentrifuge and has the largest capacity in its class.

Experiment

1. Instruments

Centrifuge: CS150NX tabletop micro ultracentrifuge
Rotor: S50A fixed angle rotor (Up to 6 tubes can be contained.)
Tube: 25PC thick-walled tube (Actual capacity: 19.8 ml), capless

2. Separation procedure

Centrifuge the infected allantoic fluid or infected cell culture medium at 6,000 rpm for 20 minutes to remove host-derived coarse foreign substances.

↓

Pour the supernatant into the new 25PC thick-walled tubes (19 ml per tube).

↓ Perform centrifugation using the S50A fixed angle rotor.
(32,000 rpm, 45 minutes, 4°C, Acc. 9, Dec. 7)

Remove the supernatant and add 1.5 ml of Veronal buffer solution including 3mM $CaCl_2$ to the sediment, then suspend again. (To minimize formation of virus clump, add a small amount of buffer solution to the sediment. Leave it on overnight at 4°C and perform pipetting.)

↓

Layer the concentrated virus fluid on 17 ml of 10 to 40% (w/v) sucrose continuous density gradient solution that is previously made in each 25PC thick-walled tube.

↓ Perform centrifugation using the S50A fixed angle rotor.
(32,000 rpm, 45 minutes, 4°C, Acc. 5, Dec. 7)

A white layer is formed slightly above the center of the tube. The virus layer can be easily observed by applying slit of light from the outside of the tube in a dark place. Collect the minimum amount of virus layer (up to about 2 ml).

↓

Dilute the fractionated virus fluid with buffer solution about 1.5 times (fluid volume after dilution: about 3 ml). Layer the diluted virus fluid on 15 ml of 30 to 60% (w/v) sucrose continuous density gradient solution that is previously made in each 25PC thick-walled tube.

↓ Perform centrifugation using the S50A fixed angle rotor.
(32,000 rpm, 3 hours, 4°C, Acc. 5, Dec. 7)

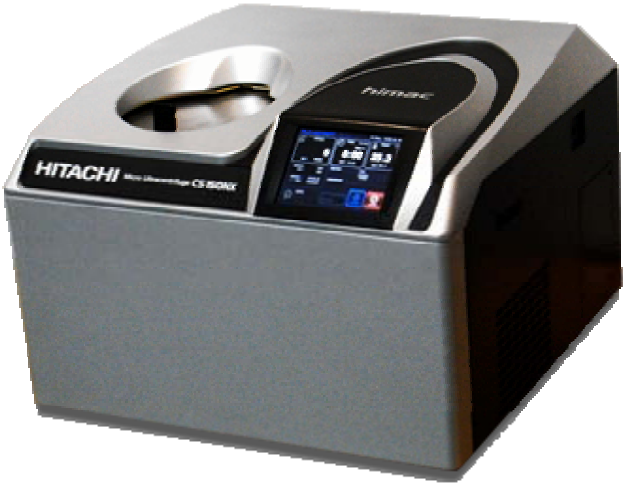
Collect the formed virus layer and dilute it with 2.5 times or more buffer solution.

↓ Perform centrifugation using the S50A fixed angle rotor.
(32,000 rpm, 1 hour, 4°C, Acc. 9, Dec. 7)

Add buffer solution to the sediment and suspend it again.

Reference: Experiment of Viruses (Particular) (Published by Maruzen Co., Ltd. in 1982)

Instrument



CS150NX tabletop micro ultracentrifuge

User-friendly T-bar handle for easy closing and opening of the rotor cover

S50A fixed angle rotor

*This rotor can also be used with the CS150NX, CS-GX ll series, CS-GXL series and CS-GX series centrifuges.

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

Hitachi Koki Co., Ltd.
Life-Science Instruments Division

1060, Takeda, Hitachinaka City Ibaraki Pref., 312-8502 Japan

Tel:(81)29-276-7384 (Dial in)

Fax:(81)29-276-7475

***The latest information is available on our web side.**
http://www.hitachi-koki.com.himac/