Separation of Influenza Viruses Using a Fixed-Angle Rotor Designed for Micro-ultracentrifuges

**Introduction**

Influenza viruses are spherical-shaped enveloped RNA viruses between 80 and 120 nm in diameter classified in Orthomyxoviridae. There are three kinds of influenza viruses depending on serotype: type A, type B, and type C. However, antigenic changes within these subtypes result in the extreme diversity of viral strains and makes an annual reformulation of the influenza vaccine necessary\(^2\). In recent years, highly virulent avian and swine flu viruses have been raising serious concerns about flu pandemics.

Following is an example protocol for the separation of influenza viruses by means of the S50-A fixed-angle rotor that is developed for the new Thermo Scientific micro-ultracentrifuges. This newly designed rotor has the largest capacity in the micro-ultracentrifuge family and allows sample processing up to 180 mL.

**Centrifuge Conditions:**

**Instruments**

All centrifuge steps were performed using the Thermo Scientific MTX 150 benchtop micro-ultracentrifuge with the S50-A fixed-angle rotor (up to 6 tubes can be contained) with 25 mL open-top polycarbonate (PC) thick-walled tubes (actual capacity: 19.8 mL).

**Separation procedures**

1. Centrifuge the infected allantoic fluid or infected cell culture medium at 6,000 rpm for 20 minutes to remove host-derived coarse foreign substances. Depending on volume, this step can be completed in a floor model superspeed centrifuge such as the Thermo Scientific Sorvall RC-6+ or a benchtop general purpose centrifuge.

2. Pour the supernatant into the 25 mL PC thick-walled tubes.

3. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 xg), 45 minutes, 4°C, Acc.9, Dec. 7

4. Remove the supernatant and add 1.5 mL of Veronal buffer solution including 3 mL CaCl\(_2\) to the sediment, then suspend again. To minimize the formation of virus clumps, add a small amount of buffer solution to the sediment. Leave it overnight at 4°C and perform pipetting to resuspend.

5. Layer the concentrated virus fluid on 17 mL of 10 to 40% (w/v) sucrose continuous density gradient solution in each 25 mL PC thick-walled tube.
6. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 xg), 45 minutes, 4°C, Acc.9, Dec. 7.

7. A white layer is formed slightly above the center of the tube. The virus layer can be observed, in a dark room, by exposing light to the tube. Collect the minimum amount of virus layer (up to about 2 mL).

8. 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 in each 25 mL PC thick-walled tube.

9. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 xg), 45 minutes, 4°C, Acc.9, Dec. 7. Collect the formed virus layer and dilute it with 2.5 times or more buffer solution.

10. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 xg), 1 hour, 4°C, Acc. 9, Dec. 7. Add buffer solution to the sediment and resuspend.

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

**Conclusion**

The protocol referenced in this brief allows the researcher to efficiently isolate viral particles through a sucrose gradient using the new fixed-angle S50-A rotor and Thermo Scientific MTX 150 benchtop or MX Series micro-ultracentrifuge. This newly developed rotor allows for the isolation of virus particles in a large volume previously reserved for standard ultracentrifuges and rotors.

**References**
