

# Separation of Influenza Virus Using the Thermo Scientific S50-A Fixed-Angle Rotor and Sorvall Micro-Ultracentrifuge

## KEY WORDS

- Influenza Virus
- Allantonic Fluid
- Cell Culture
- S50-A Rotor
- Sorvall MTX 150 Micro-Ultracentrifuge
- Sorvall MX Micro-Ultracentrifuge

## Introduction

Influenza viruses are spherical in shape with diameters ranging between 80 and 120 nm. They consist of an enveloped RNA and are classified in the Orthomyxoviridae family. 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<sup>1</sup>. In recent years, highly virulent avian and swine flu viruses have been raising serious concerns about flu pandemics.

This brief describes a protocol for the separation of influenza virus using the Thermo Scientific S50-A fixed-angle rotor and Thermo Scientific Sorvall Micro-Ultracentrifuges. This rotor provides the largest capacity in the Thermo Scientific micro-ultracentrifuge family and allows sample processing up to 180 mL.

## Procedures:

All centrifugation steps were performed using the Thermo Scientific S50-A fixed-angle rotor with 25 mL open-top polycarbonate (PC) thick-walled tubes (PN 75000610, actual volume = 19.8 mL) and the Thermo Scientific Sorvall MTX 150 Micro-Ultracentrifuge; a Thermo Scientific Sorvall MX Micro-Ultracentrifuge may alternatively be used.

## Separation Procedures

1. Remove host-derived coarse foreign substances from infected allantoic fluid or infected cell culture medium by performing centrifugation with the following parameters: 6,000 rpm for 20 minutes. *Note: Depending on volume, this step can be completed in a floor model superspeed centrifuge, such as the Thermo Scientific Sorvall RC 6 Plus, or a general purpose centrifuge.*
2. Pour the supernatant into PC thick-walled tubes. *Note: PC thick-walled tubes must be filled to a minimum of 50% capacity.*
3. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 x g) for 45 minutes at 4 °C, Acc.9, Dec. 7.
4. Remove the supernatant and add 1.5 mL of Veronal buffer solution including 3 mM CaCl<sub>2</sub> to the sediment, to minimize the formation of viral-containing clumps.



Thermo Scientific Sorvall MTX 150 Micro-Ultracentrifuge



Thermo Scientific S50-A Fixed-Angle Rotor

5. Resuspend by pipetting and leave overnight at 4 °C.
6. Layer the concentrated virus fluid over 17 mL of a 10 to 40% (w/v) sucrose continuous density gradient solution in each PC thick-walled tube.
7. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 x g) for 45 minutes, at 4 °C, Acc.9, Dec. 7.
8. 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 ~ 2 mL).
9. Dilute the fractionated virus fluid with 1.5 times buffer solution (fluid volume after dilution: ~ 3 mL). Layer the diluted virus fluid over 15 mL of a 30 to 60% (w/v) sucrose continuous density gradient solution in each PC thick-walled tube.
10. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters : 32,000 rpm (~85,800 x g) for 45 minutes at 4 °C, Acc.9, Dec. 7.
11. Collect the formed virus layer and dilute it with 2.5 times or more buffer solution to bring the total volume to 17 to 18 mL.
12. Perform centrifugation using the S50-A fixed-angle rotor with the following parameters: 32,000 rpm (~85,800 x g) for 60 minutes at 4 °C, Acc. 9, Dec. 7. Add buffer solution to the sediment and resuspend.

## Conclusion

The protocol referenced in this brief allows viral particles to be isolated efficiently through a sucrose gradients using the S50-A fixed-angle rotor and Sorvall® MTX 150 or Sorvall MX Series Micro-Ultracentrifuge. The S50-A rotor allows for the isolation of virus particles in a large volume that was previously reserved for standard ultracentrifuges and rotors.

## References

1. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (2007). US Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health.
2. Hitachi Koki Co. (2008). Separation of Influenza Viruses Using Fixed Angle Rotor Designed for Tabletop Micro Ultracentrifuge.

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