

# Separation of Rotavirus Using a Thermo Scientific Fiberlite Fixed Angle Rotor

## KEY WORDS

- Sorvall WX Ultracentrifuge
- Fiberlite Rotors
- Rotavirus
- Sorvall RC 6 Plus centrifuge
- Fecal matter

## Introduction

Rotavirus is a major pathogen of infantile gastroenteritis and continues to have a major global impact on childhood morbidity and mortality. Classified in the family Reoviridae, it is a spherical-shaped icosahedral virus approximately 70 nm in diameter and is composed of a three-layered protein capsid surrounding a genome that consists of 11 dsRNA segments. Seven rotavirus serogroups (serogroups A to G) have been described with most human pathogens belonging to groups A, B, and C.

Following is an example protocol for the separation of rotavirus by

means of the Thermo Scientific Fiberlite F65L-6x13.5 fixed angle rotor and the Thermo Scientific Sorvall WX ultracentrifuge.

## Materials and Methods

Centrifugation steps for the pretreatment of samples were performed using the Thermo Scientific Sorvall RC 6 Plus superspeed centrifuge with the Thermo Scientific Fiberlite F21-8x50y fixed angle rotor with adapters (010-0382) for the 16.0 mL Thermo Scientific Nalgene polycarbonate (PC) Oak Ridge Tubes (3139-0016). Ultracentrifugation for rotavirus isolation was performed

using the Fiberlite® F65L-6x13.5 fixed angle rotor and the Sorvall® WX ultracentrifuge with a 13.5 mL PC bottle (010-1380) and bottle assemble (010-1382); actual capacity is 10.4 mL.

## Pretreatment of sample

1. Place 1 g of patient feces in a 16.0 mL PC tube.
2. Add 9 mL of 18 MΩ water to make a 10% suspension.
3. Remove large debris and bacteria by centrifugation using the Fiberlite F21-8x50y fixed angle rotor with the following parameters: 4,000 x g for 30 minutes at 4 °C. This step can also be completed using a Thermo Scientific benchtop general purpose centrifuge.
4. Remove the supernatant to a new tube. Add an equivalent-volume of fluorocarbon and keep stirring for 2 minutes.
5. Perform centrifugation using the Fiberlite F21-8x50y rotor with the following parameters: 2,000 x g for 30 minutes at 4 °C. This step can also be completed using a Thermo Scientific benchtop general purpose centrifuge.
6. Remove the supernatant to a new tube.

## Isolation of Rotavirus

1. Using 13.5 mL PC bottles and assemble lids, layer 6 mL of the concentrated virus fluid on 4 mL of a 30% (w/w) sucrose solution.
2. Perform centrifugation in a Sorvall WX ultracentrifuge using the Fiberlite F65L-6x13.5 fixed angle rotor with the following parameters: 324,000 x g for 90 minutes at 4 °C, Acc. 5, Dec 7.
3. Remove the brown supernatant and the 30% sucrose solution with a pipette.
4. Without touching the precipitate, absorb the liquid adhered to the internal surface of the tube with Kimwipes®.



Figure 1: Sorvall WX ultracentrifuge and Fiberlite F65L-6x13.5 fixed angle rotor

5. Add 30  $\mu$ L of 18 M $\Omega$  water, cap, and incline the tube to ensure the liquid contacts the precipitate and let stand overnight at 4 °C.
6. Place the tube in a shaker and mix for 30 minutes at 4 °C.
7. Remove all the contents of the tube into a 1.5 mL micro tube.
8. Perform centrifugation at 15,000 rpm, 4 °C for 30 minutes.
9. Remove the rotavirus-contained supernatant.

## Conclusion

The protocol allows the researcher to efficiently isolate rotavirus particles using the Fiberlite F65L-6x13.5 fixed angle rotor and Sorvall WX ultracentrifuge.

## References

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