Application Note:
AN-LECF-VIRUSMU-0908

Separation of SRSV in 5 mL Tubes Using Thermo Scientific S52-ST Swinging Bucket Rotor and Thermo Scientific Sorvall Discovery M120 SE or M150 SE Microultracentrifuges

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KEY WORDS

- Virus Isolation
- Sucrose Cushion
- S52-ST Rotor
- Microultracentrifuge

Introduction

Small Round Structured Viruses (SRSVs), which cause human gastroenteritis, can be isolated via ultracentrifugation and confirmed by subsequent electron microscopy. Conventional practices require a large-scale ultracentrifuge and its applicable swinging bucket rotor for separating relatively small spherical viruses such as SRSV.

The Thermo Scientific S52-ST swinging bucket rotor spins 5 mL tubes that are traditionally used for large-scale ultracentrifuges. The S52-ST is designed for use in Thermo Scientific Sorvall Discovery M120 SE and M150 SE microultracentrifuges.

In this study, centrifugal conditions are provided for isolating human SRSV, suitable for inspection by electron microscopy. The S52-ST swinging bucket rotor and sucrose cushion effectively isolate SRSV at small volumes, and the centrifugal conditions using the equipment stated herein are also applicable to a multitude of other viruses.

Procedures

Preparation of Viral Supernatant

1. Place 1 g human stool sample into a 15 mL polypropylene centrifuge tube.
2. Add 9 mL distilled water to make a 10% suspension.
3. Centrifuge at 4,000 x g in a Thermo Scientific low-speed centrifuge with a fixed-angle rotor at 4 °C for 30 minutes.
4. Decant supernatant into a new tube; add equal amount of fluorocarbon (HCFC141b); and stir 2 minutes.
5. Centrifuge at 2,000 x g in a low-speed centrifuge as described above for 30 minutes.
6. Harvest the supernatant for ultracentrifugation or freeze if immediate use is not required.

Ultracentrifugation of Viral Supernatant

1. Place 1.2 mL of 30% (w/w) sucrose in the bottom of a 5 mL pollyallomer centrifuge tube.
2. Layer with 3.6 mL of viral supernatant.
3. Centrifuge in the S52-ST swinging bucket rotor at 38,000 rpm (147,000 x g) for 3 hours at 4 °C. Set the acceleration/deceleration profiles at 5 and 7 respectively.

Thermo Scientific Sorvall Discovery M120 SE Microultracentrifuge

Sample Clean-up

1. After ultracentrifugation, carefully remove supernatant and 30% sucrose solution with Pasteur pipet.
2. Carefully absorb any fluid deposited on internal surface of tube with a twisted piece of low-lint laboratory wiper, paying special attention not to touch the sediment.
3. Add 36 µL of purified water, making sure it covers the surface of sediment.

4. Cover tube with laboratory film and let stand overnight at 4 °C.
5. Shake tube for 30 minutes.
6. Transfer all contents of the tube to a 1.5 mL flip-top micro-centrifuge tube and centrifuge at 15,000 x g in a Thermo Scientific microcentrifuge at 4 °C for 30 minutes.
7. Remove 1 – 2 µL of supernatant and perform negative coloring to examine it under T.E.M. (transmission electron microscopy).

Conclusion

The S52-ST rotor increases swinging bucket rotor capacity nearly 2-3 fold on the Sorvall® Discovery™ M120 SE or M150 SE microultra-centrifuges. This higher 4 x 5 mL throughput permits an even broader array of applications.

Acknowledgement

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

References

Separation of SRSV in 5 mL Tubes Using ST Swinging Bucket Rotor and Discovery M120 SE or M150 SE Microultracentrifuges. Application Brief, S00318.

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