

Purification of Lambda Bacteriophage using the Thermo Scientific Fiberlite F40L-8x100 Carbon Fiber Rotor

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KEY WORDS

- Bacteriophage Purification
- Virus Concentration
- Cesium Chloride (CsCl) Gradient
- Thermo Scientific Fiberlite F40L-8x100 Rotor
- Thermo Scientific Sorvall WX Ultracentrifuge Series

Introduction^{(2), (3)}

Bacteriophage can be isolated from host cells by centrifugation. The uninfected cells are grown in culture with the virus. Several hours are required for the uninfected cells to continuously grow and divide, thereby increasing the quantities of the bacteriophage. Finally when all of the cells are infected, chemicals are added to the culture to remove the membranes of the cells. This allows the bacteriophage to be released from the cells and kept in the culture.

Traditional methods utilize swinging bucket rotors in an ultracentrifuge to concentrate virus.^{(1), (2)} The limited capacity of these rotors often required up to two working days to concentrate all the virus from 500 mL of culture solution.

Using the Thermo Scientific Fiberlite F40L-8x100 carbon fiber fixed angle ultracentrifuge rotor with the same protocol, bacteriophage from 500 mL of culture can be concentrated in one run.

Procedure

Standard 100 mL polycarbonate (PC) tubes can be used in the rotor for the initial sedimentation of the virus at 37,000 rpm for 1.5 hrs. After the virus is collected from the large tubes, smaller tube volumes can be used for the final purification of the virus using cesium chloride (CsCl) gradients.⁽³⁾ Instead of using a smaller rotor, centrifuge tube adapters can be utilized in the Fiberlite® F40L-8x100 rotor cavities to accommodate smaller tubes or the standard polycarbonate

bottles can be run half filled.⁽⁴⁾ Place the tube adapters for the Fiberlite rotor and centrifuge the samples in the Thermo Scientific Sorvall WX ultracentrifuge overnight at 37,000 rpm for 16 to 18 hours at 4° C.

Discussion

Purified bacteriophage particles can be collected and stored to be used later for preparing bacteriophage DNA.⁽³⁾ This DNA can be used for cloning and genetic studies. This technical note demonstrates the versatility of carbon fiber rotors and explains how sample preparation times can be greatly reduced using the Fiberlite F40L-8x100 rotor.



Figure 1: Thermo Scientific Fiberlite F40L-8x100 carbon fiber ultracentrifuge rotor

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