

Purification Of Lambda Bacteriophage using the F40L-6x100 mL FIBERLite® Carbon Fiber Rotor

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

In previous studies the Beckman rotors SW 27 (6 x 39 ml) and the Type 50 Ti (8 x 13 ml) rotors were used in the Ultra centrifuge to concentrate the virus (spinning the rotor at maximum speeds). This study took approximately two working days to concentrate all the virus from 500 mL of culture solution.

In this study the Beckman ultracentrifuge was used with a single FIBERLite F40L-6x100 mL carbon fiber rotor. The rotor is manufactured by FIBERLite centrifuge, Inc., Santa Clara CA and it was used to concentrate the bacteriophage from the same amount of culture solution.

When standard 100 ml tubes were used in the rotor for the initial sedimenting of the virus the rotor was spun at 40,000 rpm for two hours. After the virus was collected from the large tubes smaller tube volumes were required for the final purification of the virus. Instead of using a smaller rotor, centrifuge tube adapters were utilized in the F40L-6x100 mL rotor cavities to accommodate the smaller tubes.

The tubes were placed in the tube adapters for the FIBERLite® rotor and centrifuged in the ultracentrifuge overnight at 40,000 rpm for 16 to 18 hours at 4 0 C. using cesium chloride gradients.

Purified Bacteriophage particles can be collected and stored to be used later for preparing bacteriophage DNA. This DNA can be used for cloning and genetic studies.

This Applications Note showed the versatility of FIBERLite® carbon fiber rotors. A single rotor can be operated at maximum speeds even when using adapters to reduce sample volumes for duplicating studies done with metallic rotors. Sample preparation times will also be greatly reduced.

REFERENCES

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- Maniatis, T. E.F, Fritsch, J. Sambrook, (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory , Cold Spring, New York.



Rotor Model: F40L-6x100

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