

Multiple Thermo Scientific Fiberlite Rotors Used for Purifying DNA from Cleared Lysate in the Thermo Scientific Sorvall Evolution RC Superspeed Centrifuge

Owen Mitch Griffith, Ph.D.

KEY WORDS

- Plasmid DNA Isolation
- Superspeed Centrifuges
- Carbon Fiber Rotors
- Density Gradient
- Cesium Chloride
- Bacterial Pelleting

Introduction

Multiple types of centrifuges are found in the laboratory environment, and may differ in specifications including size, speed, and volume capabilities. Large capacity floor model centrifuges allow for the isolation of biological particles from cell cultures or homogenates (pelleting) found in volumes up to 3,000 mL to 6,000 mL. The speeds for pelleting can vary in ranges of 0 to 15,000 rpm (low-speed) and 0 to 26,000 rpm (super-speed). Lower volume applications such as the clarification or purification of subcellular particles can also be completed in these floor model centrifuges at volumes as little as 96 mL, total capacity. Furthermore, ultraspeed and microultra centrifuges are capable of speeds 30,000 rpm and greater (up to 150,000 rpm) and are used to isolate macro-molecules like nucleic acids, viral particles, nanoparticles, etc. For basic micro volume applications at speeds up to 21,000 rpm, microcentrifuges provide a cost effective and convenient alternative to the larger floor models.

The Thermo Scientific Sorvall Evolution RC superspeed centrifuge can accommodate the full range of lightweight, corrosion-resistant Thermo Scientific Fiberlite carbon fiber rotors, which range from micro to large scale volumes (96 mL to 6000 mL total capacity).

This technical note describes a procedure using three Fiberlite™ rotors F8S-6x1000y, F21S-8x50y, and F21S-48x1.5/2.0 for the isolation of plasmid DNA from cleared lysate, all in a single superspeed centrifuge.

Procedure

Culture Preparation

Inoculate Luria-Bertani broth (LB) with *Escherichia coli* (*E. coli*) con-



The Thermo Scientific Sorvall Evolution RC Centrifuge is available with a wide selection of Thermo Scientific Fiberlite Carbon Fiber Rotors.

taining the plasmid pBR322 and incubate overnight at 37°C with vigorous shaking.

Bacterial Pelleting

Add cultured samples to 1 L polycarbonate or polypropylene Thermo Scientific Nalgene superspeed bottles with special sealing cap and place in the Fiberlite F8S-6x1000y mL large volume carbon fiber rotor.

Centrifuge the sample at 8,500 rpm (15,860 x g) in the Sorvall® Evolution™ RC centrifuge to pellet the bacteria at a set run time of 10 min^{1,2}. Alternatively, smaller volume culture samples may be pelleted using many of the carbon fiber rotors, such as the F9S-4x1000y mL rotor, in the Evolution RC superspeed centrifuges with the conditions described.

Lysate Clarification

To produce crude lysate, the bacterial pellet can be lysed using an alkali or boiling method¹. The smaller Fiberlite F21S-8x50y rotor with Bioseal technology can be used to clarify the crude lysate from the harvested bacteria cells. Collect approximately 400 mL of crude lysate and centrifuge at high speeds of 20,500 rpm (49,892 x g) for approximately 30 min using the Sorvall Evolution RC centrifuge.

DNA Purification

Adjust approximately 50 mL of the cleared lysate solution with 6.58 M cesium chloride/ethidium bromide (CsCl/EtBr, $P = 1.80$ g/mL). Using a Pasteur pipette, layer 0.5 mL of this solution below 0.5 mL Tris-HCl, and

10 mM EDTA pH 8.0 in 1.5 mL microcentrifuge tubes. Place these tubes in the Fiberlite F21S-48x1.5/2.0 mL fixed-angle rotor and spin at 20,000 rpm (42,560 x g) in the superspeed centrifuge for 16 hours to collect the plasmid DNA³.

Results

The Evolution RC centrifuge can virtually be three centrifuges in one.

As described above, this instrument can spin the high-capacity Fiberlite 6-liter rotor at approximately 15,860 x g (8,500 rpm) to harvest bacteria or yeast cultures grown in fermenters.

The Fiberlite F21-8x50y rotor with Bioseal technology can be used to clarify the crude lysate at 49,892 x g (20,500 rpm), eliminating the need for an additional floor model centrifuge capable of greater speed and force.

The final purification step to collect plasmid DNA can be done using the Fiberlite F21S-48x1.5/2.0 mL rotor instead of an ultracentrifuge rotor and unit.

Conclusion

The versatility of the Evolution RC centrifuge and the comprehensive selection of Fiberlite carbon fiber composite rotors allow three separation methods to be accomplished in one centrifuge, making it a cost-effective and space-savings solution for biological research laboratories today. Furthermore the F21-8x50y rotor with Bioseal technology with enhanced biocontainment features allows for the maximum protection of your samples.

Rotors featuring Bioseal technology have been rigidly tested for microbiological containment by the Public Health Laboratory Service, Centre for Applied Microbiological Research, Porton Down, UK, and shown to be suitable for use with materials up to ACDP Category 3 as categorized by the Advisory Committee on Dangerous Pathogens.

References

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The Fiberlite F8S-6x1000y mL carbon fiber rotor



The Fiberlite F21S-8x50y mL carbon fiber rotor with Bioseal technology

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North America:
USA / Canada
+1 866 984 0039

Europe:
Austria
+43 1 801 40 0

Belgium
+32 2 482 30 30

France
+33 2 2803 2000

Germany national toll free
08001-536 376

Germany international
+49 6184 90 6940

Italy
+39 02 02 95059 341

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+31 76 571 4440

Nordic countries
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Russia / CIS
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China
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+91 22 6716 2200

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+81 45 453 9220

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+852 2885 4613

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