Technical Note:
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Pelleting Bacteria Using Thermo Scientific Fiberlite Large Volume Rotors

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KEY WORDS

- Bacterial Pelleting
- Carbon Fiber Rotors
- Superspeed Centrifuges

Figure 1: Thermo Scientific Fiberlite F10-4x1000 LEX carbon fiber rotor.

Figure 2: Thermo Scientific Fiberlite F12-6x500 LEX carbon fiber rotor.

Figure 3: Thermo Scientific Fiberlite F8-6x1000y carbon fiber rotor.

Introduction

Bacterial cells grown in tissue culture media for extracting nucleic acids are frequently harvested with conventional superspeed centrifuges using metal, fixed-angle rotors, which carry bottles with volumes up to 500 mL.[1,2] These rotors range in weight from 40 to 49 lbs. (19 to 22 kg). The set run time to pellet the bacteria and solids in these heavy metal rotors can be up to 20 minutes per run, while the total run time ranges from 35-45 minutes per run, including time to accelerate up to speed and decelerate from maximum speed to rest.

Thermo Scientific Fiberlite large volume carbon fiber rotors are used with standard 500 mL and 1000 mL bottles to sediment solids such as bacteria, yeast and other protein precipitates from fermenters or bioreactors. These rotors typically range in weight from 19 to 35 lbs. (9 to 15 kg).

The new large-volume Fiberlite® carbon fiber rotors, the Fiberlite F12-6x500 LEX and Fiberlite F10-4x1000 LEX rotors, are the lightest rotors of their kind at 19 lbs. (9 kg) and 21 lbs. (10 kg) respectively.

Due to the reduced weight of the Fiberlite large-volume carbon fiber rotors, superspeed centrifuges like the Thermo Scientific Sorvall RC-6 Plus and Evolution RC centrifuges can sediment large volumes of solids in a total run time of 8 to 10 minutes. When considering the time required for acceleration and deceleration, the lighter weight rotors allow for the larger volume samples to experience the maximum force for a longer period of time resulting in a more efficient separation in less time.

This technical note describes sedimentation protocols for bacterial cells in Fiberlite large volume carbon fiber rotors (Fiberlite F8-6x1000y, F10-4x1000 LEX, and F12-6x500 LEX) and compares the run times to equivalent metal rotors.

Procedures

Inoculate Luria-Bertani broth (LB) with *Escherichia coli (E. coli)* containing plasmid, pBR322, and incubate overnight at 37° C with vigorous shaking.[1, 3, 4]

Polycarbonate or polypropylene bottles can be used in all 3 rotors. Fill the bottles with the bacteria-containing medium and place in the appropriate rotor.

Place the carbon fiber rotor in the superspeed centrifuge and spin under the conditions shown in Table 1. In all studies, the pellets to be obtained will be compact and easy to remove after the supernatant is decanted from the bottles.

Rotors	Max. Speed (RPM)	Max. G-force (x g)	Volume (mL)	Empty Weight (kg)	Set Run Time (min)
Thermo Scientific Fiberlite F12-6x500 LEX	12,000	24,500	3,000	9	8
Thermo Scientific Fiberlite F10-4x1000 LEX	9,500	16,900	4,000	10	10
Thermo Scientific Fiberlite F8-6x1000y[1]	8,500	15,800	6,000	15	10
Thermo Scientific Sorvall GS-3	9,000	13,700	3,000	22	20
Thermo Scientific SLA-3000	12,000	24,000	3,000	14.4	20
Competitor Rotor X	10,000	17,700	3,000	18	20
Competitor Rotor Y	10,000	18,500	3,000	12	15

Table 1: Rotors, set run times and total run times at maximum speeds for *E. coli* pelleting.
[1]Rotor exclusively available for the Sorvall® Evolution RC centrifuge.

Results:

The bacterial counts were obtained and showed that the overnight culture had approximately 1.7 ± 2.75 x 10^{10} counts/mL. The supernatants after centrifugation for 5.0 minutes, had an average count of 2.1 ± 1.93 x 10^{7} counts/mL. In all cases, the supernatants appeared very clear by visual inspection.

When the counts in the supernatant was greater than 1 x 10^{8} counts/mL for each centrifugation, the run was repeated for 3 to 7 minutes longer. The counts were approximately 1.65 x 10^{6} counts/mL after a total run time of 8 to 10 minutes. This run time included 90 seconds acceleration and deceleration times for the Fiberlite F12-6x500 LEX rotor and 120 seconds acceleration and deceleration times for the Fiberlite F10-4x1000 LEX rotor.

Conclusion

The suggested set run times for the conventional superspeed centrifuges is 8 minutes with the Fiberlite F12-6x500 LEX rotor and 10 minutes with the Fiberlite F10-4x1000 LEX rotor or Fiberlite F8-6x1000y rotor. However, the run times can be up to 20 minutes when the Thermo Scientific Sorvall GS-3 and SLA-3000 metal rotors and 2 competitor metal rotors are used.

The table above suggests a substantial increase in throughput efficiency when using carbon fiber rotors. Not only is the pelleting processed decreased in time, but larger volumes of sample can be processed per run. This is made possible by the lightweight Fiberlite carbon fiber rotors. In addition, the lighter weight Fiberlite rotors make it easier to remove rotors from the centrifuge for storage or cleaning when these rotors are used in a multi-use or academic laboratory. The lighter weight of carbon fiber rotors may also reduce the amount of inertia the centrifuge motor must overcome to turn a Fiberlite rotor. The lower stress on the motor relates to less wear and tear on critical drive components such as mounts, bearings, shafts, and seals allowing for a reduction in centrifuge maintenance costs.

References

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