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FIBERLite Vortex 21K Centrifuge Used For Purifying Plasmid DNA From Cleared Lysate

Owen Mitch Griffith, Ph.D

Traditionally, three types of centrifuges are found in the laboratory environment: a large capacity floor model centrifuge designed to isolate biological particles from cell cultures or homogenates found in volumes of 3,000 ml to 6,000 ml of solution at speeds ranging from 0 – 6,000 rpm, a high or Superspeed centrifuge designed for medium speed (0 – 21,000 rpm) applications to clarify or purify subcellular particles in volumes ranging from 400 ml to 3,000 ml, and an ultra speed centrifuge for speeds of 30,000 rpm and greater to isolate macro molecules like nucleic acids. The new Vortex 21K centrifuge, manufactured by FIBERLite Centrifuge Inc., Santa Clara, CA, combines capacity to process samples volumes ranging from 40 ml to 6,000 ml with special applications and the mechanical ability at 21,000 rpm to purify macro molecules like plasmid DNA from clear lysates obtained from cell cultures of bacteria.

FIBERLite Vortex 21K Centrifuge

Methods

Luria-Bertani broth (LB) was inoculated with Echerichia coli (E-coli) containing the plasmid pBR322 and incubated overnight at 37 degrees C with vigorous shaking. The one-liter polycarbonate bottles manufactured by Nalgene *Nunc* a Division of Thermo Fisher Scientific, Rochester N.Y., were used in the F8-6 x 1000y mL large volume rotor. The sample was centrifuged at 8,000 rpm (15,700 x g) for a total run time of 20 min to pellet the bacteria at a set run time of 10 minutes. (1, 2).

The small F21-8 x 50 rotor was used to clarify the crude lysate from the lysed bacteria cells. Approximately 400 ml of the crude lysate was collected from the 6000 ml of culture and centrifuged at high g-forces of 52,000 x g (21,000 rpm) in approximately 30 min using the same Superspeed Vortex 21K centrifuge.

Approximately 50 ml of the cleared lysate solution was adjusted with 6.58 M cesium chloride-ethidium bromide (1.80 g/ml density). A Pasteur pipette was used to layer 0.5 ml of this solution below 0.5 ml Tris-HCl, and 10 mM EDTA pH 8.0 in 1.0 ml tubes of the FIBERLite Fixed angle rotor F21 -48x1.0 ml. The rotor was also centrifuged at 21,000 rpm in the Vortex 21 centrifuge for 16 hr to collect the plasmid DNA (3).

Conclusion

The FIBERLite Vortex 21K centrifuge is virtually three centrifuges in one. As shown above, this instrument will spin the high capacity FIBERLite 6-liter rotor at 14,000 x g (8,000 rpm) to harvest bacteria or yeast cultures grown in fermenters as previously done in a Low Speed High Capacity Centrifuge. The FIBERLite F21-8x50 was then used to clarify the crude lysate at 52,000 x g (21,000rpm), eliminating the need for an additional floor model centrifuge. The final purification step to collect the Plasmid DNA was done using the F21-48x1.0 mL rotor instead of an ultracentrifuge rotor. The versatility of Vortex 21K instrument 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 solution for Biological Research laboratories today.

References:

1. Maniatis, T., Fritsch, E.F., and Sambrook, J., Molecular Cloning 90-93 Cold Spring Harbor Laboratory, New York (1982).
2. Bolivar, F., Rodriguez, R.L., Betlach, MC., and Boyer, H.W., Gene 2, 75-93 (1977)
3. Garger, S.J, Griffith O.M., and Grill L.K. Biochemical and Biophysical Research Communications Vol 117, No. 3, 839-842, (1983)

For Further Information:

Contact us at:

FIBERLite Centrifuge, Inc.
422 Aldo Ave.
Santa Clara, CA 95054
408-988-1103 Tel
408-988-1196 Fax
www.piramoon.com