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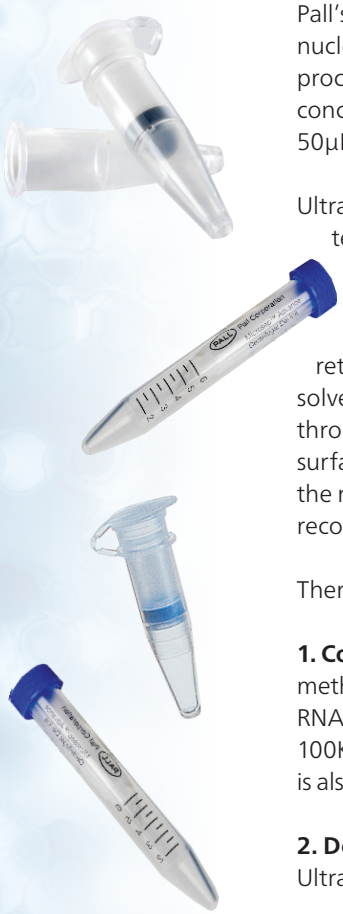
Centrifugal Devices:
Simplifying Nucleic Acid and Protein Sample Prep

PALL Laboratory



Pall's centrifugal devices simplify many common nucleic acid and protein sample preparation procedures. These devices provide efficient concentration and salt removal of samples from 50µL to 60mL in just minutes.

Ultrafiltration (UF) is a membrane separation technique based on molecular size, although other factors, such as molecule shape and charge, can also play a role. Molecules larger than the membrane pores in the UF membrane will be retained at the surface of the membrane while solvent and smaller solute molecules can freely pass through. This molecular exclusion at the UF membrane surface leads to concentration of the protein solute in the retained fraction (the retentate) and can be recovered from above the membrane.

There are three generic applications for ultrafiltration:

1. Concentration. Ultrafiltration is a very convenient method for the concentration of dilute protein or DNA/RNA samples. It is gentle and does not shear DNA up to 100Kb or cause loss of enzymatic activity in proteins. It is also very efficient, typically offering > 90% recovery.

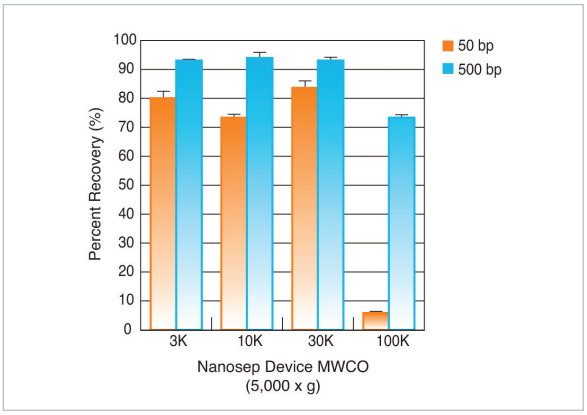
2. Desalting and Buffer Exchange (Diafiltration). Ultrafiltration provides a convenient and efficient way to remove or exchange salts, remove detergents, separate free molecules from bound molecules, remove low molecular weight components, or rapidly change the ionic or pH environment.

3. Fractionation. Fractionation using ultrafiltration is effective in applications, such as the preparation of protein-free filtrates, the separation of unbound or unincorporated label from DNA and protein samples, and the purification of PCR products from synthesis reactions.

Centrifugal devices can replace traditional separation techniques, such as column chromatography, preparative electrophoresis, alcohol or salt precipitation, dialysis, and gradient centrifugation when performing the following:

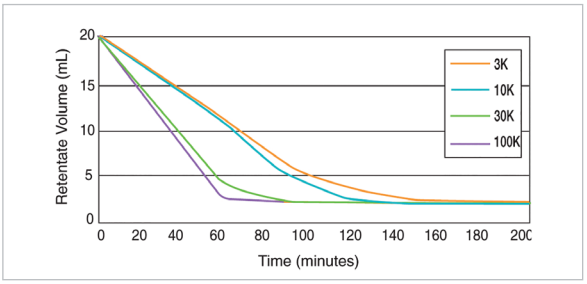
- Protein or nucleic acid concentration
- Desalting
- Buffer exchange
- Fractionation of protein mixtures
- Separation of primers from PCR products
- Separation of labeled nucleic acids or proteins from unincorporated nucleotides
- Virus concentration or removal
- Clarification of cell lysates and tissue homogenates

DNA Recovery as a Function of Device MWCO



A 500µL sample of a 100µg/mL DNA fragment solution containing 50 and 500bp double-stranded DNA fragments was centrifuged at 5,000 x *g* in Nanosep devices to a final volume of 50µL. Recovered samples were quantified using absorbance at 260nm. The 100K device was able to differentiate between the sizes of the DNA fragments.

Macrosep Advance Centrifugal Devices: Reduced Spin Time



Protein solutions were processed in each of the Macrosep Advance devices. Average time (minutes) is plotted against mL of remaining product to be filtered using a swinging bucket rotor at 5,000 x *g*. Solutions are 3K: Protamine Sulfate, 0.1% in 1X PBS, 10K: Cytochrome C, 0.025% in 1X PBS; 30K: IgG, 0.1% in 1x PBS; and 100K: Apoferritin, 0.1% in 1X PBS.

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