

CONCENTRATION OF PROTEINS

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Materials/Reagents/Equipment	Vendor
Reagents	
Protein to be concentrated	
Materials	
5, 10 or 30 K MWCO concentrators as needed	Orbital Biosciences, Viva Science
Equipment	
Sorval Biofuge <i>primo R</i>	Kendro Laboratory Products

Procedure

1. Select the proper molecular weight cut off (MWCO) for the protein concentrator. As a general rule the pore size of the concentrator membrane should be two and a half times smaller than the molecular weight of the protein (i.e. if your protein is 30 kDa, use 10 kDA MWCO). Concentrator volumes come in 500 μ l (use microcentrifuge), 4 ml, 7 ml, 15 ml, and 20 ml samples. Select the size according to your needs. Add some buffer to the concentrator and spin it to condition and rinse the membrane.
2. Depending on the total amount of protein, take a small sample and set it in your concentrator. Spin at the recommended speed for the particular concentrator for 15 – 20 minutes. Take out the protein and perform Bradford. If losses are higher than 30%, do Bradford on the flow through. If protein is detected in the flow through, it may be that the unit is defective or a smaller MWCO should be used. If the protein precipitates try increasing/decreasing NaCl concentration or use additives such as detergents or glycerol that may help stabilize the protein. *Generally, when the protein precipitates or the percentage loss is great without increasing concentration, you should proceed to Optimum Solubility Screen.* If the protein does not precipitate, then proceed with concentrating.
3. If the ability of the protein to be concentrated is not known, the concentration should proceed in incremental steps in order to avoid over-concentration and precipitation. Once you establish the concentration conditions, add more protein to your concentrator. Once the volume is reduced and the desired concentration is achieved, **do not add** more protein. **Take out the concentrate by pipetting it up and down and transfer it to a new microfuge tube.** Add more protein sample to your concentrator for another round of concentration. Combine the concentrated protein for further concentration if needed.