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Concentrating samples for protein identification

It is often the case that the volume of a protein sample prepared by a micro purification procedure is much larger than that can be loaded for gel electrophoresis. If the total amount of proteins in such sample is very low, concentrating the sample to a volume that can be loaded to 1-2 gel lanes (10-30 ul total) is probably the easiest way to increase the chance to detect proteins on a gel and to identify protein by us.

However, tradition methods for concentrating protein such as membrane dialysis, TCA precipitation and membrane filtering are often not applicable in micro purification procedures due to large percentage of sample loss. Drying down protein samples will lead to high concentration of salt any other chemicals that may interfere with gel electrophoresis.

Based on our experience, we suggest a simple and rapid method in which proteins are bound to some reverse phase beads packed in a micro-column, washed to remove salt, and elute with SDS loading buffer (with or without boiling) before loading on gel. We have been using GeneClean from Stratagene and R1/R2 from Applied System and each method offers a good sample recovery. Some other micro-scale, protein-concentrating methods also have been reported to effectively concentrate protein sample without significant protein loss. Eluting proteins from an affinity column with an SDS-PAGE sample-loading buffer can reduce sample volume but more non-specific, bound proteins may show up.

However, since each protein has its unique properties and different purification procedures may result in different samples, researchers are strongly encouraged to determine and optimize their best sample concentrating procedures. In principle, the way of concentrating sample will have no effect on the subsequent MS protein identification step for a gel separated protein. So the effectiveness of a sample concentrating method can be easily judged by sample recovery rate and behavior of such sample during gel electrophoresis, which can be monitored by running a SDS PAGE loaded with pre-concentrated and post-concentrated samples at the same amount.

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