

Depletion Methods for Plasma or Serum Biomarker Studies

Comparative proteomic studies on plasma and serum are often performed by 2-D electrophoresis or 2-D DIGE. Finding biomarkers in plasma and serum is difficult, however, because the 10 to 12 most abundant proteins account for over 85% of the protein mass. Thus, using 2-D gel electrophoresis to detect differences in levels of low-abundance proteins which may be biomarkers of disease, is not possible from crude serum or plasma. A common first step in many 2-D DIGE-based biomarker discovery projects is to deplete the serum or plasma of serum albumins, IgG, and other abundant proteins.

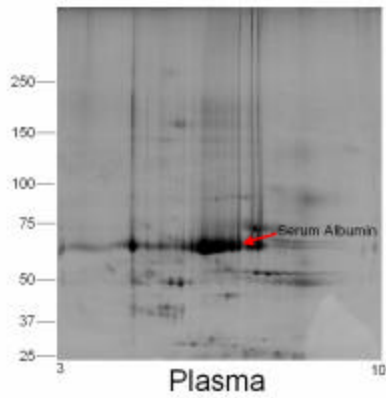
Since we have been involved in a number of these studies, we have evaluated several depletion methodologies, and we therefore thought it would be helpful to offer some suggestions on which technologies we have found to be easiest to use and give the best results. Currently in the UNC-Duke Proteomics Center, we are using the Agilent Multi-Affinity Removal Spin Cartridge HU-6 column for our depletion studies. This column targets human serum albumin, IgG, IgA, transferrin, haptoglobin, α 1-antitrypsin. It is relatively quick and easy to use. Moreover, the high-salt buffer disrupts most protein/protein interactions occurring in native serum or plasma. This reduces the chances that low-abundance biomarker proteins will be captured along with serum albumin or other proteins during the depletion procedure, which reduces one of the major "pitfalls" of depletion procedures. One drawback to the high salt buffer used in this protocol, however, is that the samples must be de-salted by dialysis, size-exclusion chromatography, or reversed-phase chromatography before a 2-D gel can be run. Thus, more time is involved in the sample cleanup procedure, and there is some chance for sample loss.

Another kit we have used successfully is the Genway Mix-12 kit which targets HSA, IgG, fibrinogen, transferrin, IgA, IgM, high-density lipoprotein (Apo A-I & Apo A-II), α 1-antitrypsin, ? 1-acid glycoprotein, IgY, α 2-macroglobulin. While targeting more potentially-interfering proteins, this type of immunoaffinity sample cleanup methodology uses a lower salt buffer during the depletion procedure. Thus it may not disrupt protein/protein interactions as well, which might lead to the loss of protein biomarkers which co-immunopurify with the target proteins. Samples processed this way, however, require less post-procedure cleanup before 2-D gel electrophoresis.

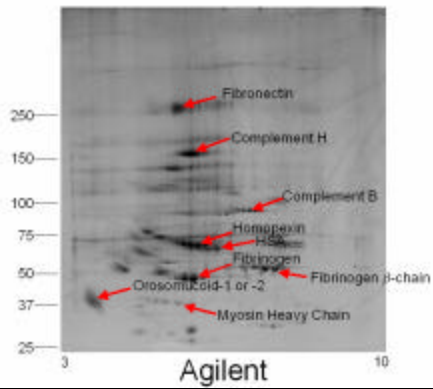
It is impossible to recommend a particular affinity-based method, however, because different suppliers have different matrices which target different proteins and, of equal importance, different manufacturers use different buffer compositions which may affect depletion efficiencies. One benefit of immunoaffinity-based protocols, however, is that many manufacturers will custom mix affinity matrices for a investigators particular application. This can be used to customize the depletion protocol for a particular application, to optimize the recovery of a particular biomarker protein once it has been identified.

The current trend in biomarker discovery, however, is not to use the 2-D gel and depletion approach, but to digest the proteins into peptides and to reduce the complexity of the samples by improving the separation of the components so that depletion is not necessary. Usually this is done with multidimensional LC, most often with a combination of ion exchange and reversed-phase LC separation techniques. This allows the analysis of complex samples such as plasma, where proteins are present in a wide dynamic range, without removing any of the proteins, and without discriminating against low-solubility proteins such as membrane proteins. At the UNC-Duke Proteomics Center, we are developing methods based on multidimensional liquid chromatography coupled to our 12 Tesla Bruker Apex Qq-FTICR.

Our approach is to reduce and alkylate a pair of serum or plasma samples, and then to digest them with trypsin. The peptides from each sample are then differentially-labeled using either acetic anhydride or hexadeuteroacetic anhydride. When the samples are mixed and injected into the multidimensional LC, this differential isotopic labeling gives a characteristic 3.007 Da difference between heavy- and light-labeled samples which is easily detected in the FTICR spectra. The presence of 3 deuterium atoms in the acetyl group does not dramatically alter the ionization or detection of the labeled peptides compared to acetyl groups with hydrogens. Therefore, to detect differences in protein abundance, the peak intensities of each 3.007 Da doublet is compared. Thus with, this unbiased approach, we can detect and identify proteins that change in abundance between the two samples.



2-D gel of undepleted plasma.



2-D gel of plasma depleted with the Agilent kit, and desalted with a size-exclusion microconcentrator.