

ProteoSep

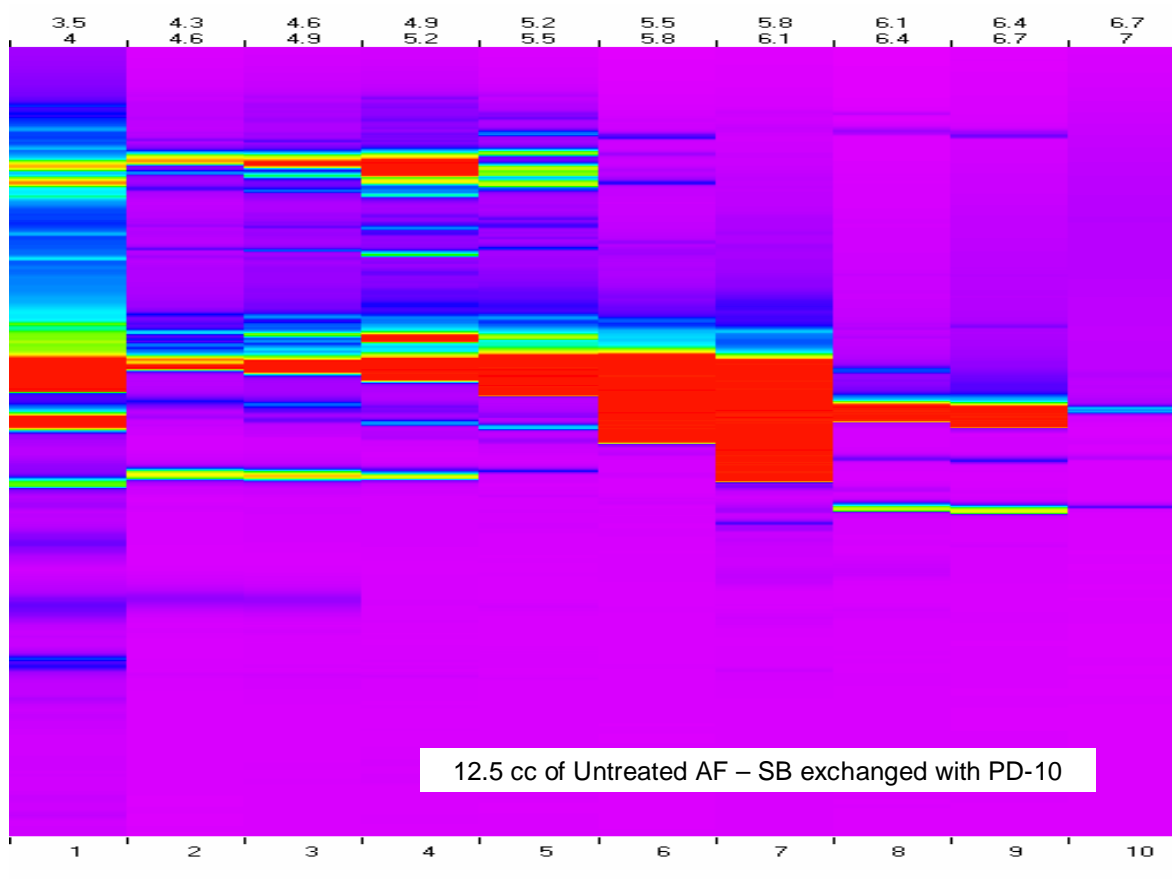
Tech Note #2 – Efficient Processing of Dilute Protein Samples: The Analysis of Amniotic Fluid

Dilute protein samples like Amniotic Fluid (AF) can be processed untreated or through EtOH precipitation techniques using the ProteoSep chemistries.

Conclusion: Cold EtOH precipitation followed by SB resolubilization is an excellent way to concentrate and solubilize dilute protein samples such as AF, Urine, Ascites or other dilute protein biofluids.

2D Liquid phase processing of more dilute protein samples can be tedious unless you can easily concentrate or isolate the sample proteins for ProteoSep analysis.

The ProteoVue map shown below highlights the results for untreated AF where several aliquots of the pooled AF sample were PD-10 exchanged followed by multiple injections onto the CF column to load the complete sample (3 x 5 cc Injections onto the CF column prior to running the CF gradient method.)



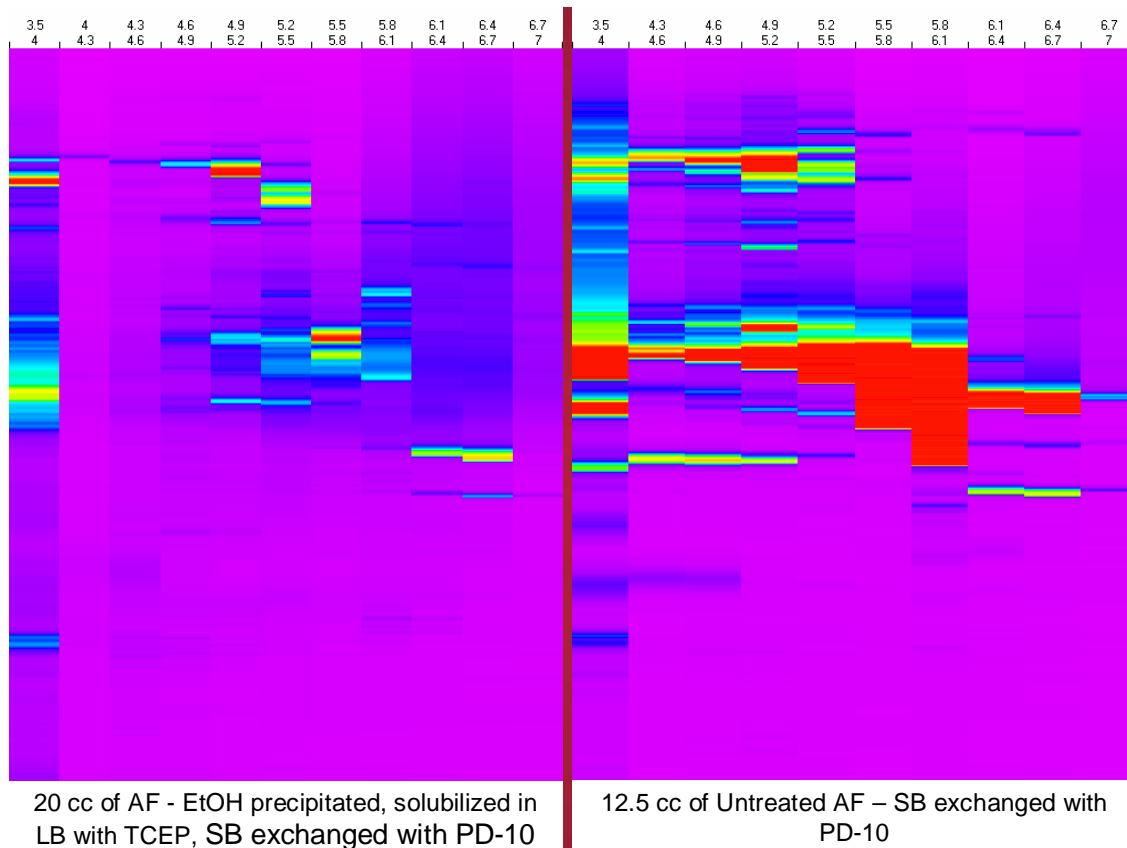
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To simplify and speed up the AF analysis procedure we investigated the use of cold EtOH precipitation as a means of improving the overall process of isolating and reconstituting dilute protein samples like AF for ProteoSep analysis.



The left ProteoVue map shows the results obtained for a ProteoSep run on the same pooled AF patient sample (20 cc) but was first Ethanol (EtOH) precipitated and the resulting precipitate resolubilized in standard Lysis Buffer (LB) containing TCEP reducing agent followed by a PD-10 exchange protocol. Analysis of the supernatant using the 2D HPRP column showed that no protein was left in the EtOH fraction. The right map is the same map as the full image map highlighted above (12.5 cc of AF processed only by exchanging with a PD-10 column into SB). **Note the “TCEP Effect” described in Tech Note #1.**

Having observed again that AF, like other albumin containing biofluids, cannot be treated with TCEP we did a 2D separation on two different pooled AF patient samples (7.5 cc each) which were cold EtOH precipitated and resolubilized in 2.5 cc of SB and PD-10 exchanged. The amount of AF that you can process is not limiting but it is recommended that the maximum amount to protein processed for the ProteoSep 2D separation be 5-10 mg total protein per PF2D run.

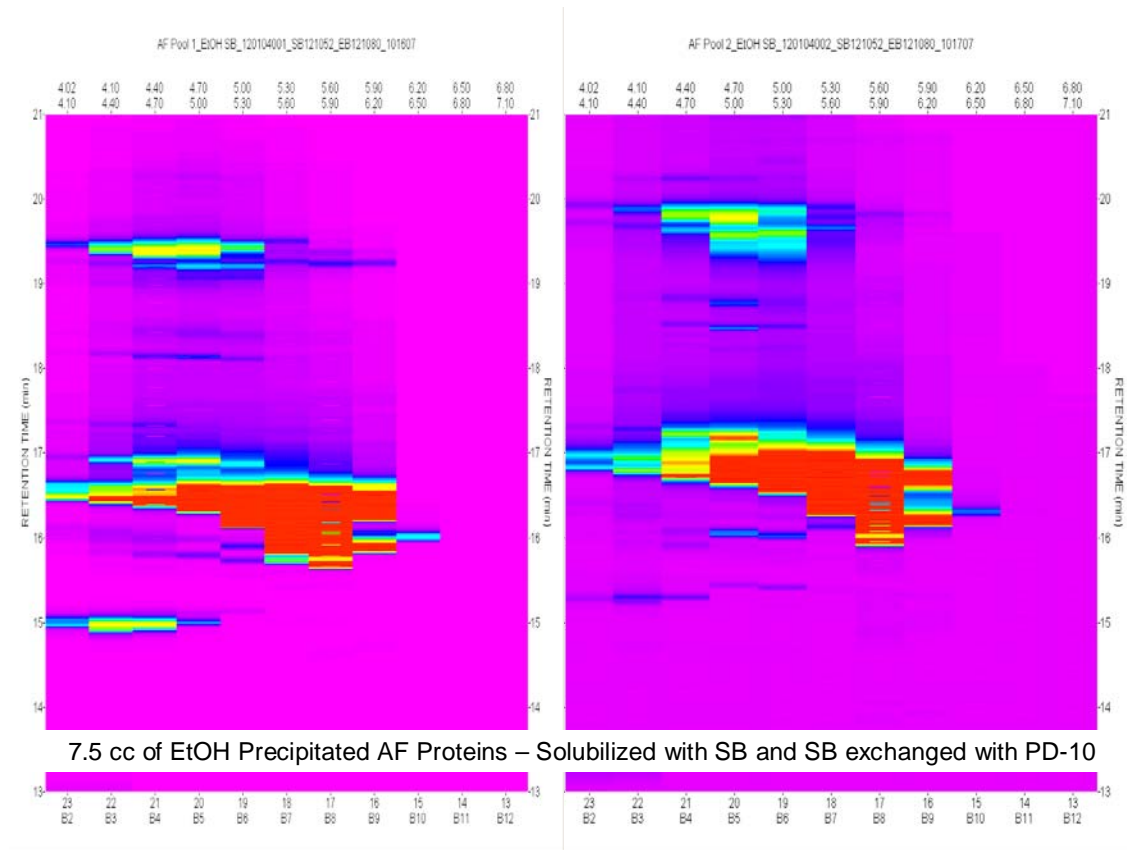
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Below are the ProteoVue maps for the two different Pooled AF patient samples. The differences noted in the images are a reflection of the differences in the patient populations that were pooled for comparative analysis.



General Procedure for EtOH precipitation of dilute protein biofluids

1. In a centrifuge tube, precipitate the proteins from AF by adding cold, absolute ethanol in a 1:3 (v/v) sample to ethanol ratio (for example, 9mL of ethanol for a 3mL sample volume). Vortex and allow the mixture to stand on ice or in a refrigerator at 4°C for 30min.
2. Centrifuge the mixture at 3000 g for 30 minutes at 4°C and discard the supernatant. Store the pellet at –20 °C until ready for use [–80 °C for long term (>2 weeks) storage].
3. Resolubilize the Pellet with 2.5 cc of SB and PD-10 exchange with 3.5 cc of SB. Multiple pellets can be combined to a recommended maximum of 5-10 mg of total protein in the 2.5 cc of SB for PD-10 exchange.
4. Dilute the PD-10 eluent to 5.0 cc for injection onto the PF2D.

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