

# ProteoSep

## Tech Note #5 – Optimum CF operation.

1. Proper Handling and Storage of ProteoSep Start Buffers (SB), Eluent Buffers (EB) and Chromatofocussing (CF) Columns.
  - Let the SB and EB warm to room temperature (RT) on a bench-top away from sunlight. Sonication or heating in a Hot Water bath to speed up the warming process can damage and will reduce the lifetime of the EB! Properly warmed SB allows for much faster water washed CF column equilibration times: 0.3 mL/min flow rate for 60 minutes.
  - Avoid leaving the SB and EB remain at RT overnight after a CF run as this will reduce the lifetime of the buffers, in particular the EB. Store SB and EB at 4 – 8°C. Do not freeze buffers!
  - Following proper handling and storage procedures will avoid excessive additions of iminodiacetic acid (IDA) to the EB to maintain a pH of 4.0 for CF fractionations. Excessive addition of IDA will cause warping of the pH slope in the pH 5 – 4 region of the CF profile.
  - Flush new HPCF columns with 20 mL of HPLC grade Water prior to SB equilibration. CF columns can be stored in HPLC Grade water on the PF2D for several days with no adverse affects. Store CF columns for long periods in 10-20% (v/v) isopropanol/HPLC Water.
2. Proper pH Electrode Handling and Storage.
  - Calibrate the pH Electrode before every CF Run. A properly working electrode will have pH slope values between 97% and 103%. Electrodes with calibrated pH slope values significantly outside the 97% and 103% range require soaking in either the Beckman pH electrode storage solution or in a 3M KCl solution for 24 – 48 hours. If proper slope ranges cannot be achieved after this procedure, a new electrode is probably needed.
  - For best results, fill the electrode holding cell to the top with DI water and then slowly and carefully insert the calibrated electrode with twisting until the electrode is fully inserted. [Use a paper towel to catch the overflow of water.] This ensures that no air bubbles are trapped in the pH cell or connecting lines and it helps in proper operation of the pressure regulator.
  - Immediately after completion of the CF run remove the pH electrode and store in either of the electrode storage solutions outlined above.

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## 3. High Ionic Strength Solution (HISS) wash composition for Proteins with $pI < 4.0$

- A 1M NaCl solution containing 0.2% (w/v) n-octyl- $\beta$ ,D-glucopyranoside (n-OG) is the recommended HISS wash composition for routine use as the 3<sup>rd</sup> solvent in the CF fractionation. This HISS composition is a “pressure” friendly wash that has been demonstrated to improve recovery of highly acidic and some highly retained proteins from the CF column. This is especially important where higher sample loads are desired and for samples containing a large amount of a dominant protein such as albumin (e.g. serum).

*This HISS wash composition has been extensively tested in our labs and is based on data published by Oliver Barre & Marc Solioz of the Department of Pharmacology, University of Berne, Switzerland ([marc.solioz@ikp.unibe.ch](mailto:marc.solioz@ikp.unibe.ch)) in: Proteomics 2006 (6) 5096-5098 and it is gratefully acknowledged.*

- The HISS wash composition – 30% n-propanol 1M NaCl – is more effective as a clean-up solution after multiple fractionations to help increase longevity of the HPCF Column. This wash composition will effectively remove any residual hydrophobic sample components that may build up on HPCF (and HPRP) columns over time.

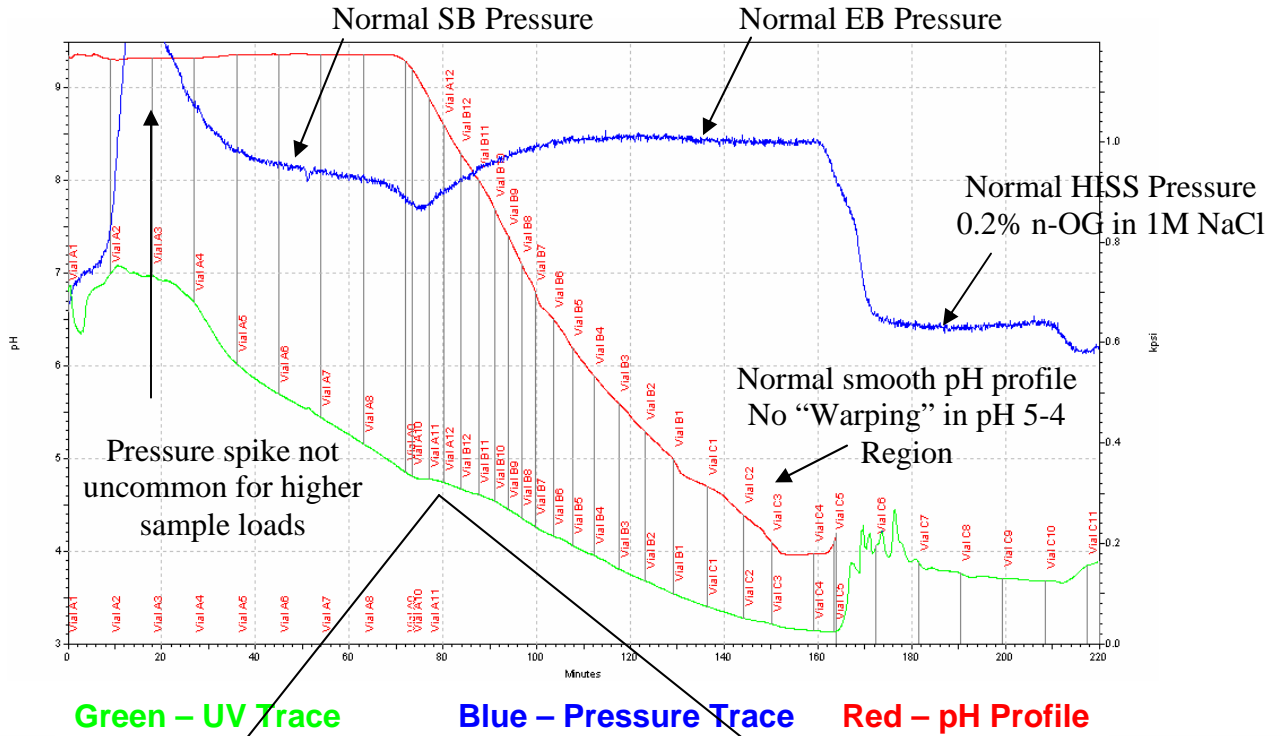
## 4. Basics for Optimum CF Profiles

- 5 cc sample injections are optimum. Dilute samples to 5 cc with SB whenever possible prior to injection. Larger volumes of more dilute samples are always better than smaller volume of more concentrated ones for optimum CF sample loading and fractionation.
- A proper pH profile should end at the measured pH for the EB (pH ~ 4) and show no significant warping in the pH 5 - 4 range. The pH profile will typically reach the final EB pH in 120 – 150 min. **Remember, PF2D CF fraction collection is based on pH, not retention time (RT).** Shifts in CF peak RT for SB – EB/HPCF column combinations are to be expected. Proteins, however, will be collected in the proper pH fraction, regardless of where they elute in time with the PF2D.
- If the final pH of a CF Run ends much higher than the measured pH of the EB and the pressure profile is normal, the pressure regulator is likely malfunctioning and should be checked for leaks or replaced.
- UV 280 is sub-optimal for protein detection. A CF profile may show no protein, but HPRP analysis at UV 214 shows significant protein (see below).

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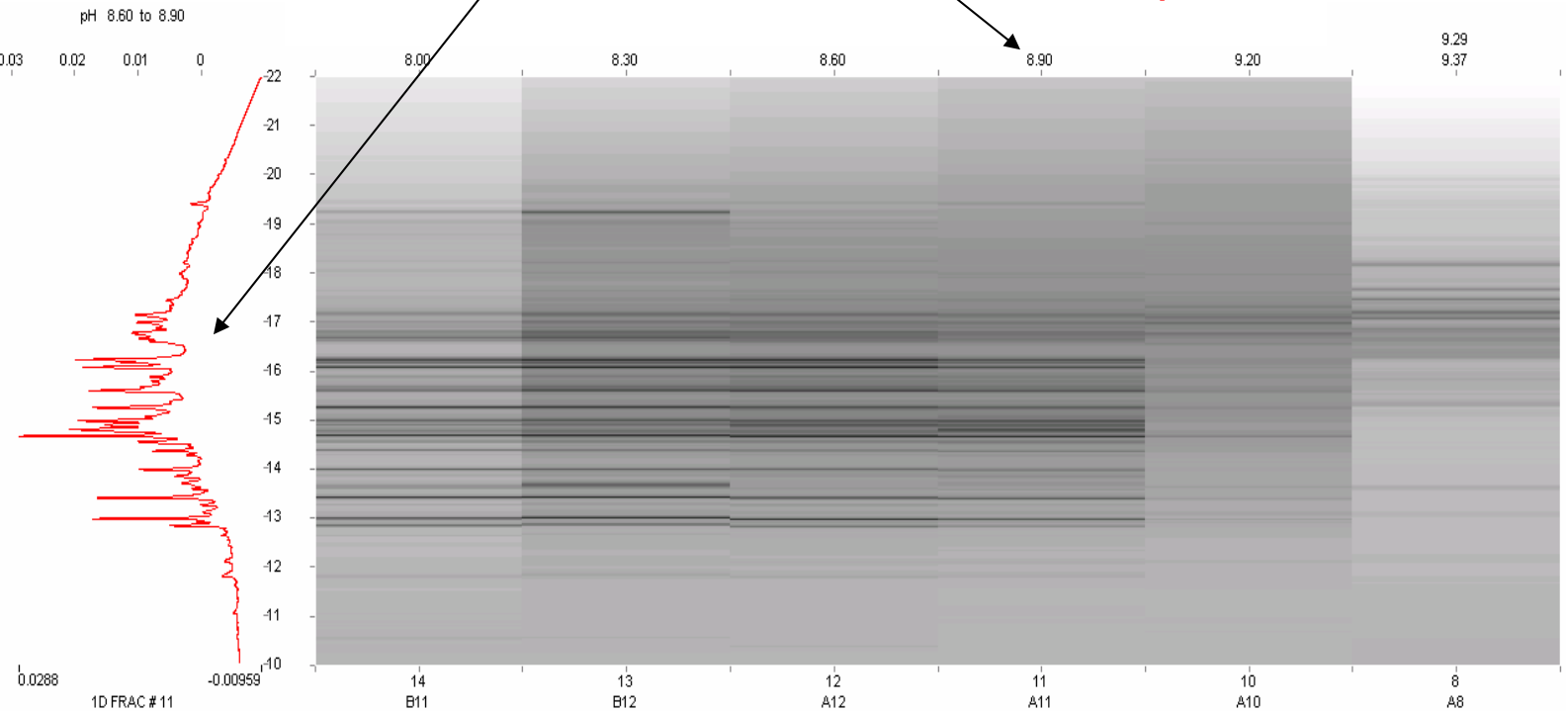
## CF Profile of a 5 mg sample of 10 Day old Mouse Embryos Lysate



Green – UV Trace

Blue – Pressure Trace

Red – pH Profile



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