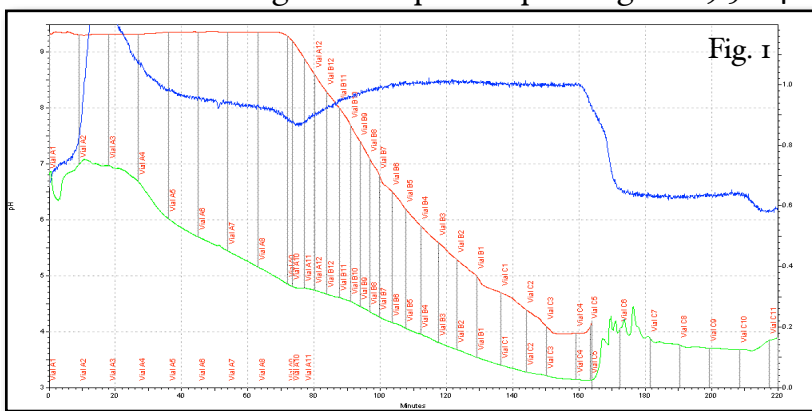# Start and Eluent Buffers for Extended pH range
# CF Fraction collection from pH 9.5 – 4.0*

Figure 1: ProteomeLab PF2D CF profile for a 5 mg lysate sample of 10 day old Mouse embryos using a New ProteoSep SB & EB combination allowing for an expanded pH range of ~9.5 – 4.0

Fig. 1

| Green: UV Trace | Blue: Pressure Trace | Red: pH Profile |

Figure 2 below illustrates the full ProteoVue map for this CF sample. As is evident, this particular sample has a large majority of its expressed proteins in the very basic region of the CF profile. There are a large number of low expressed proteins in this region with pH > 8.3 (Figure 3). An expanded pH range is very useful when searching for unique basic proteins in this sample. The standard ProteoSep Buffers (pH range 8.5 – 4) would not "fractionate" proteins in this pH range as they would be lost in the initial SB wash in the profile.
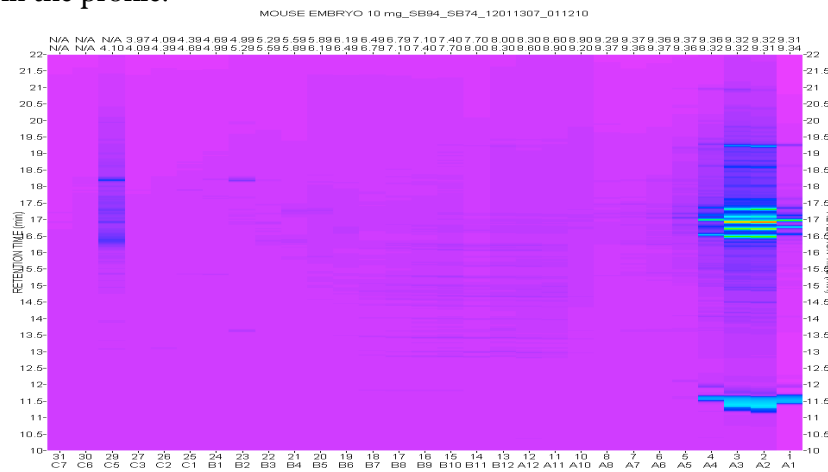
The operating pH range for standard ProteoSep Start and Eluent buffers (SB & EB) in Chromatofocussing (CF) fractionation has been between pH 8.5 – 4. While this has pH range is satisfactory for many types of samples, there are numerous occasions where an expanded range is desired to fractionate more basic proteins known to be present in the sample. To address this challenge we have investigated many new combinations of buffer components and additives for the SB and EB formulations. The goal was to match buffers with the ProteoSep CF column for expanded pH range operation, compatible with the operation of the PF2D instrument, and to maintain an ideal $\Delta pH/\Delta t$ CF gradient.

MOUSE EMBRYO 10 mg_SB94_SB74_12011307_011210

Figure 2: Full 2D Map Profile of a 5 mg sample of 10 Day old Mouse Embryos Lysate

Efficient and high resolution ProteoSep CF operation requires that the ΔpH/Δt gradient be in the range of 0.05 – 0.1 pH units/minute. Too slow of a pH gradient can make for dilute samples and long run times, and too fast of a pH gradient generates poor separation and too small of sample volumes for useful 2nd dimension HPRP analysis. This ΔpH/Δt rate range translates to a proper pH slope time range to be between 45 – 90 minutes: start of the pH drop to the end of the pH drop. This requires an end-time between 115 – 160 minutes for the pH to reach the final EB pH of 4.0 in the CF profile for any SB – EB/CF column combination in the standard default Beckman ProteomeLab PF2D method. 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 always be collected in the proper pH fraction, regardless of where they elute in time.
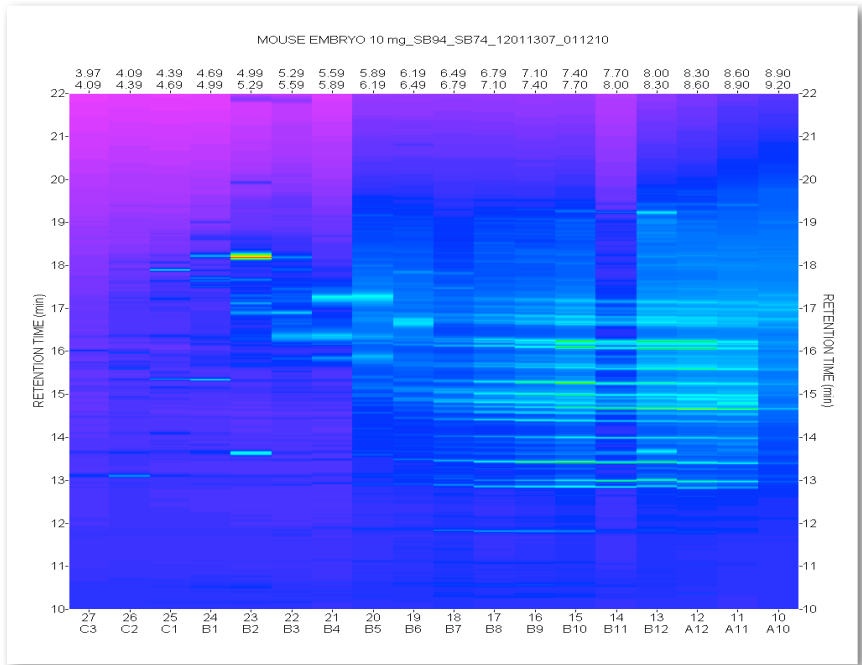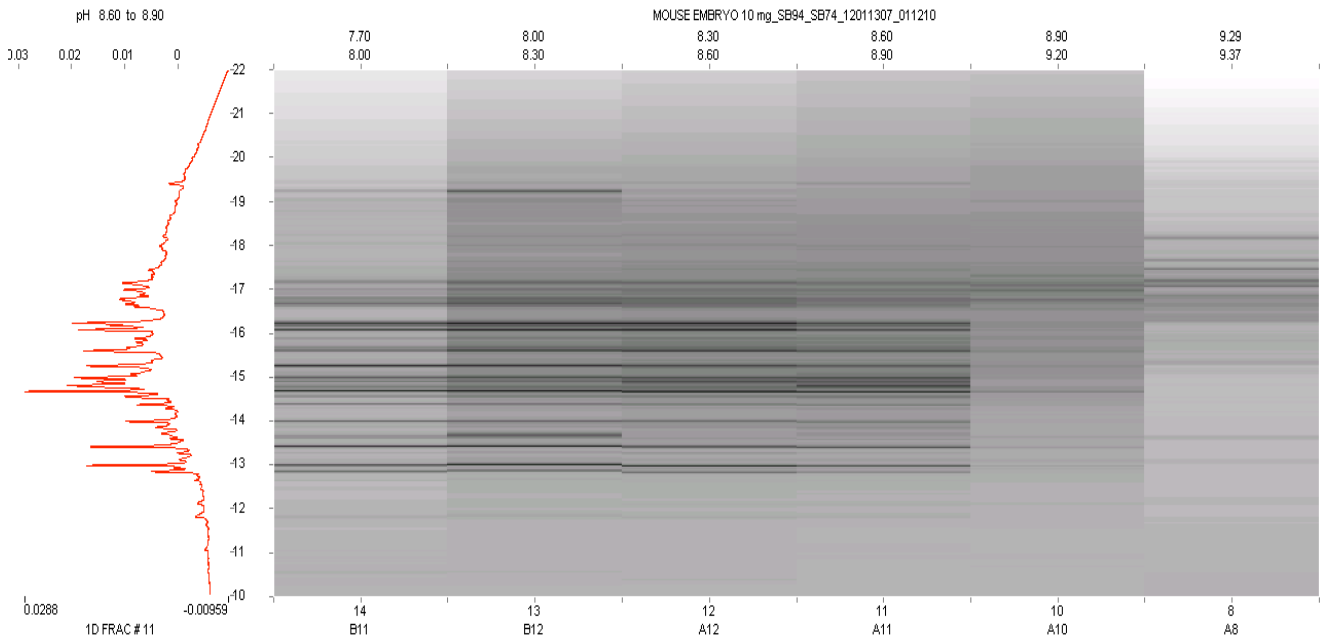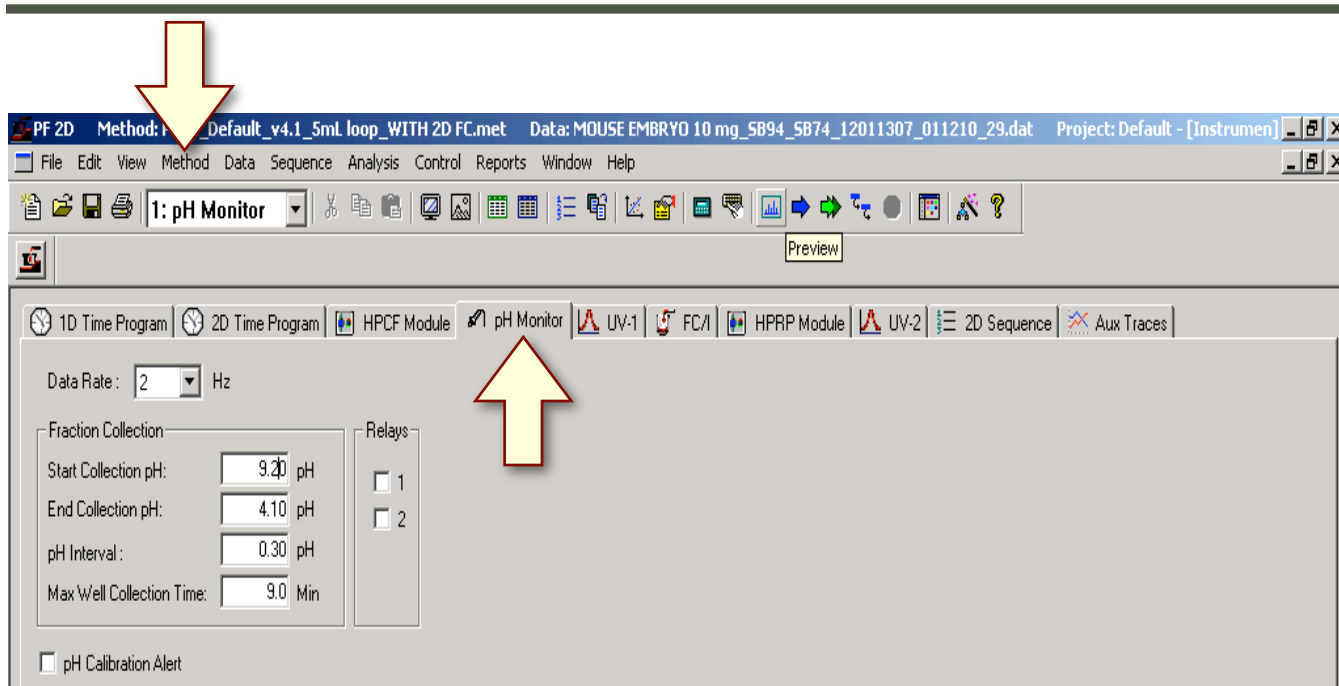

Figure 3. Profile of a 5 mg sample of a 10 Day old Mouse Embryo Lysate – pH 9.2 – 4.0

For PF2D users using ProteoSep CF Runs using the expanded pH range Buffers:

1. Adjust the pH of the SB to 9.5 – 9.6 using NH4OH.

2. Adjust the ProteomeLab PF2D method by going to **Method/Instrument Setup** and press the pH monitor Tab.

3. Input **9.2** in the Start pH collection Box and **4.1** in the End pH collection Box.

4. The minimum CF fraction volume for a default 250 uL 2D HPRP injection volume using any pH interval input value is 400 uL. Using shorter interval values (e.g. 0.2) may require changing the default injection volume for the 2D analysis in the FC/I tab.

5. Max Well Collection Time input value depends on the 96 Well plate volume used for the CF run.

6. Press "Apply" and save the method after completion of the new input values

*The expanded pH range Buffer combination is an extension of numerous publications by Prof. David M. Lubman and his group at the U of Mich. Medical Center, specifically; Zheng, S., Schneider, K.A., Barder, T.J., Lubman, D.M., Biotechniques 2003 (35) 1202-1212. More Lubman group publications can be found on our website.

Expanded Range ProteoSep Buffer Part numbers.

ProteoSep Start Buffer ER – A59670

ProteoSep Eluent Buffer ER – A59675