

## Monitoring Development of Chromatographic Methods With the Experion™ Automated Electrophoresis System

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### Introduction

Gel electrophoresis is the standard method for evaluating results from chromatography experiments, but even with modular precast gel and buffer systems, a great deal of hands-on work, time, and laboratory space is required to obtain results. These limitations are compounded by the effort required to produce data in a format suitable for laboratory notebooks, meetings, presentations, and the electronic archival systems used by many companies to support their regulatory compliance programs. These challenges create serious bottlenecks for all protein chemists, but especially for process developers who perform many chromatography runs a day, with each run requiring time-consuming electrophoretic analysis of multiple fractions.

The Experion automated electrophoresis system and Pro260 analysis kit can be used to accelerate the process of developing a purification scheme for an antibody or other protein. The Experion system combines electrophoresis, staining, destaining, detection, basic analysis, and digital result documentation into a single automated step, allowing analysis of up to ten protein samples in as little as 30 min. The Experion system provides rapid analysis of column fractions, including protein sizing, quantitation, and purity information, enabling the scientist to make accurate decisions regarding the purification steps in real time. Data can be shared immediately in presentations, analysis, or discussion, which enables process developers to quickly move on to the next phase of experimentation. Moreover, with the validated US FDA 21 CFR Part 11-compliant features of the Experion Security Edition software, the electronic data can be easily archived or submitted to the FDA.

In this tech note we examine the data generated in a 3-day workshop to illustrate how the Experion system fits into the process development workflow. During the workshop, students were divided into teams and provided with a murine IgG<sub>1</sub> monoclonal antibody (mAb) previously purified over a protein A affinity resin. Their task was to identify and optimize an appropriate chromatographic purification method using hydrophobic interaction, ion exchange, and/or ceramic hydroxyapatite media. We present some of the results from one group that developed a purification process using the BioLogic DuoFlow™ chromatography system and CHT™ ceramic hydroxyapatite Type I support. The Experion system was used to evaluate antibody purity and yield in various column fractions.

### Methods

#### Antibody Purification

Workshop participants were provided with a murine IgG<sub>1</sub> mAb that had been isolated from a cell culture supernatant derived from cell line AE-1 (Invitrogen Corp.). The mAb had been purified as follows: A 5 ml HiTrap column connected in series with a MabSelect SuRe protein A column (both from GE Healthcare) was equilibrated with 50 mM Tris, 4 M NaCl at pH 9. The mAb-containing sample was titrated to pH 6.5 with 0.5 M NaH<sub>2</sub>PO<sub>4</sub> and loaded onto the column. The antibody was eluted with 0.1 M glycine, 50 mM NaCl, pH 3 and then neutralized with 1 M Tris, pH 9 and 10 mM NaH<sub>2</sub>PO<sub>4</sub>.

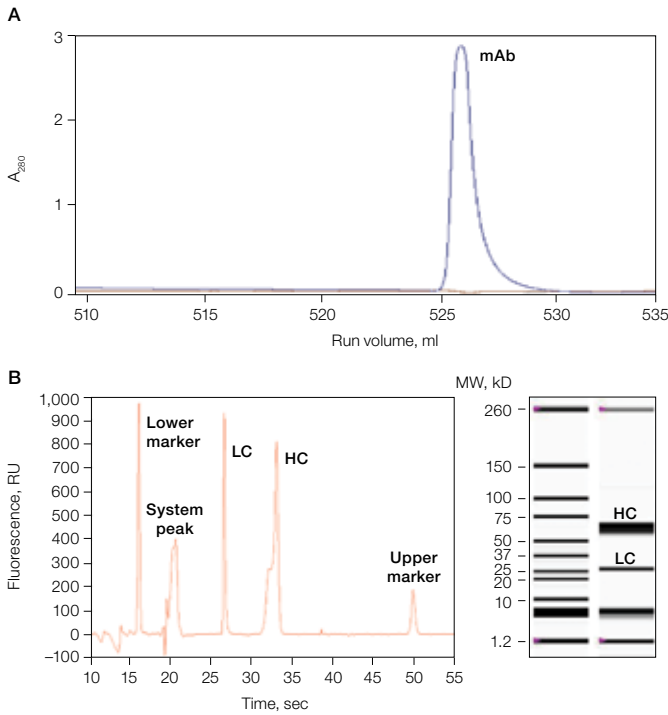
Participants then applied the protein A-purified mAb over a CHT ceramic hydroxyapatite, Type I, 20 μm column equilibrated with 10 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 6.5. The column was washed with 2 column volumes of 10 mM NaH<sub>2</sub>PO<sub>4</sub>, and the antibody eluted using a linear salt gradient to 10 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 M NaCl, pH 6.5. To check the quality of the purified product, size exclusion chromatography was then performed on a 24 ml Superdex 200 column (GE Healthcare) in 2x PBS containing 2 M urea, pH 7.2. All separations were performed on a BioLogic DuoFlow system.

## Experion Pro260 Analysis

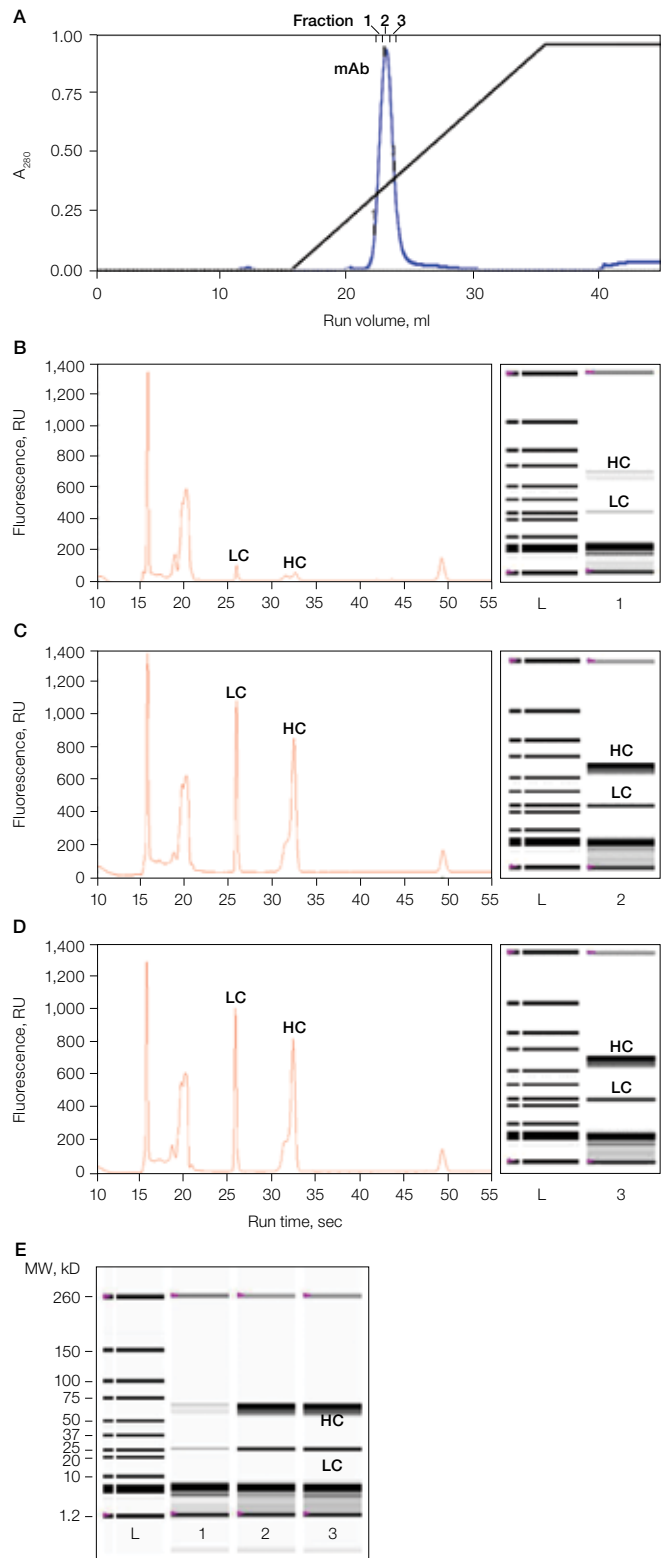
Experion Pro260 analysis kits include Experion Pro260 protein ladder, sample buffer, gel solution, fluorescent stain, spin filters, and microfluidic chips. Reducing and nonreducing sample buffers were prepared by mixing 30  $\mu$ l of Experion Pro260 sample buffer stock with either 1  $\mu$ l of  $\beta$ -mercaptoethanol or with 1  $\mu$ l of deionized water, respectively. Antibody samples were prepared under both reducing and nonreducing conditions by mixing 4  $\mu$ l of sample with 2  $\mu$ l of the appropriate sample buffer, heating for 5 min at 95°C, and then diluting with 84  $\mu$ l of 0.2  $\mu$ m filtered water. Prepared samples were then loaded onto chips that were primed according to the protocol described in the Pro260 analysis kit instruction manual. Initial mAb samples (protein A-purified) required dilution in 1x PBS buffer prior to Experion analysis, while samples taken at various time points during CHT purification were used directly, without dilution.

## Results and Discussion

Development of process- and research-scale mAb purification procedures can be significantly accelerated with online detection tools for quantitation and qualification of samples. In this tech note we demonstrate how use of the Experion system can accelerate workflow and process development.

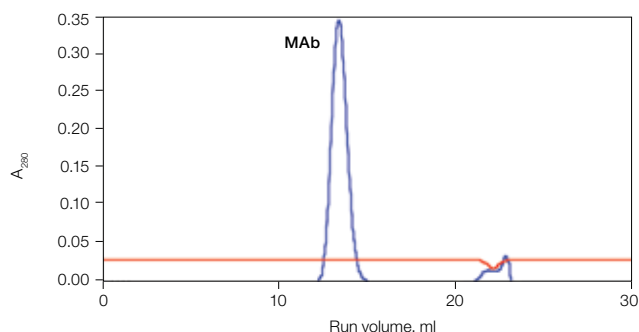


**Fig. 1. Protein A purification of murine IgG, mAb.** **A**, chromatogram generated by Biologic DuoFlow software, showing the protein A-captured monoclonal IgG; **B**, Experion Pro260 analysis showing the electropherogram (left) and virtual gel image (right) of the eluted protein fraction under reducing conditions. Left lane of gel image, Pro260 protein ladder; right lane, mAb-containing fraction.



**Fig. 2. CHT purification of protein A-purified murine IgG, mAb.**

**A**, chromatogram generated by Biologic DuoFlow software showing the CHT-purified antibody. Three fractions, designated 1, 2, and 3, were chosen for Experion analysis. Blue trace,  $A_{280}$ ; black trace, NaCl gradient. **B–D**, Experion Pro260 analysis showing the electropherograms (left) and virtual gel images (right) of the eluted protein fractions 1 (B), 2 (C), and 3 (D). Left lane of gel image, Pro260 protein ladder; right lane, mAb-containing fraction. **E**, composite gel image comparing all three fractions.



**Fig. 3. Size exclusion chromatography of purified protein.** Note the symmetrical protein peak, which indicates that the CHT-purified mAb is free of contaminants and aggregates.

A murine IgG<sub>1</sub> mAb was purified in a two-step process using protein A and ceramic hydroxyapatite media. At each step of the purification scheme, a sample was run on an Experion Pro260 protein chip. Experion software generated an electropherogram, simulated gel image, and results table for each sample.

The mAb was first captured on a MabSelect SuRe protein A column (Figure 1A). A sample of the eluted peak was analyzed with the Experion system under reducing conditions and showed peaks at 27 kD and 66 kD, representing the light chain (LC) and heavy chain (HC) of the antibody (Figure 1B). The heavy-chain peak showed broadening at 60 kD, which was possibly the result of the different glycosylation states of the heavy-chain protein.

The protein A-captured antibody was then passed over a CHT ceramic hydroxyapatite column to further purify the mAb and to remove antibody aggregates, leached protein A, DNA, and lipopolysaccharides (Figure 2A). Three fractions taken during elution of the peak were analyzed by the Experion system, also under reducing conditions, and showed peaks at 25 kD and 64 kD, representing the light chain (LC) and heavy chain (HC) of the antibody (Figure 2B–E). The Experion analysis, from sample preparation through the chip run, was achieved in 30 min, much faster than the 2–3 hr generally required for SDS-PAGE.

As a further method of quality control, a sample of the CHT-purified mAb was analyzed by size exclusion chromatography (Figure 3), which showed a symmetrical peak, indicating that the mAb was free of contaminants and aggregates.

## Conclusions

Experion Pro260 analysis is comparable to traditional SDS-PAGE in terms of performance (Chang et al. 2005, Zhu et al. 2005) but is a more rapid analysis that complements the need for quick data acquisition during process development. Experion analysis, from sample preparation through the chip run, is achieved in as little as 30 min compared to the 2–3 hr generally required for SDS-PAGE. Experion software automatically calculates the molecular weight of each resolved protein in a sample and provides information regarding the relative sample concentration and purity of each sample. The optional Security Edition software offers tools for compliance with US FDA 21 CFR Part 11 regulations.

## References

- Chang B et al., The Experion system: microfluids-based automated electrophoresis, Bio-Rad bulletin 5285 (2005)
- Zhu K et al., Performance comparison of the Experion automated electrophoresis and SDS-PAGE for protein analysis, Bio-Rad bulletin 5299 (2005)



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