

Automation of CE-SDS Sample Preparation for PA 800 *plus* CE-SDS Assays

Using the Biomek 4000 Laboratory Automation Workstation

Capillary SDS gel electrophoresis (CE-SDS) has been widely adopted by the biopharmaceutical industry for analysis of therapeutic proteins, many based on monoclonal antibodies (MAbs). Manual sample preparation for CE-SDS analysis is time-consuming and remains a potential source of operator variation and human error.

Beckman Coulter's PA 800 *plus* Pharmaceutical Analysis System (Figure 1) was designed to maximize the advantages of capillary electrophoresis (CE) and has become the industry standard for CE-SDS analysis. Although the PA 800 *plus* replaces the manual process of pouring and running slab gels, sample preparation must still be performed manually. Automation of the manual steps on the Biomek 4000 Laboratory Automation Workstation (Figure 1) ahead of CE-SDS analysis by the PA 800 *plus* resulted in reduced hands-on time and elimination of potential operator variability while delivering a robust assay.

The sample preparation for CE-SDS analysis of MAbs on the PA 800 *plus* requires denaturation of the proteins by heating the samples in detergent, either in the presence or the absence of a reducing agent. To run the prepared samples, the PA 800 *plus* requires vials in the inlet and outlet buffer trays be filled with various kit buffers and this process is a potential source of human error. The sample and buffer trays are then loaded into the PA 800 *plus* for analysis (Figure 2).

The Biomek 4000 Workstation was used to automate the sample and buffer preparation for the IgG Purity and Heterogeneity Assay kit from Beckman Coulter. The automation method drives the denaturation/reduction of 1 to 24 samples (with optional normalization) as well as transfer of the PA 800 *plus* working buffers to buffer vials. Adapter plates were designed to hold the PA 800 *plus* buffer trays on the deck of the Biomek 4000 Workstation (Figure 1). Results with two different reducing agents showed excellent consistency in peak migration time and peak area across experiments with no cross contamination between samples.



Figure 1. Biomek 4000 Workstation, adapter plate for buffer trays and PA 800 *plus*.

Discovery
in motion.

 **BECKMAN
COULTER**

Life Sciences

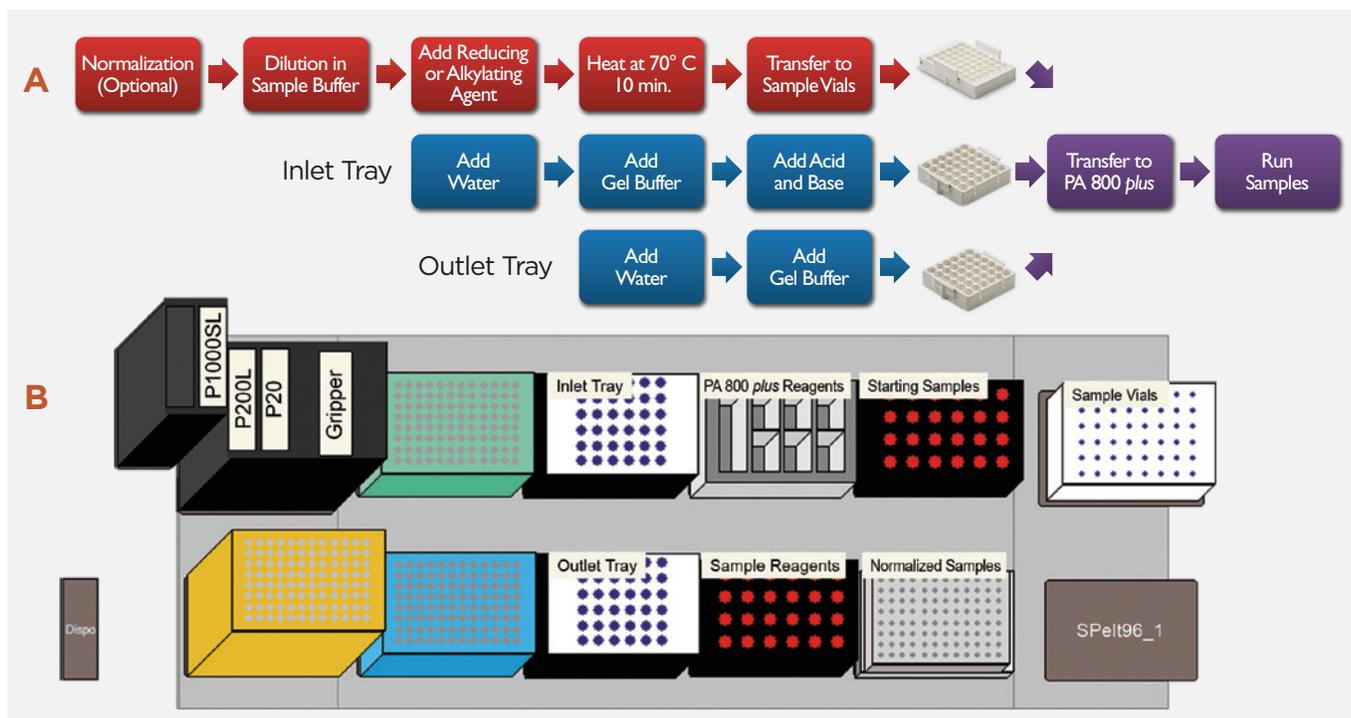


Figure 2. A. Process Workflow. The Biomek 4000 Workstation is used to automate the preparation of samples (red) and buffer trays (blue). The user transfers the sample tray and buffer trays to the PA 800 plus and runs the samples (purple). B. Biomek 4000 Workstation deck layout.

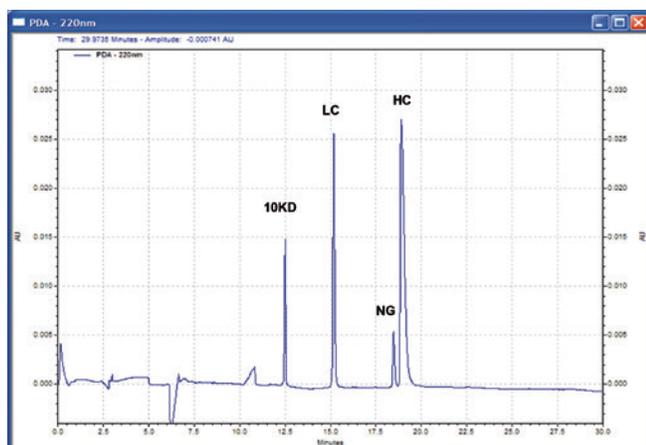


Figure 3. Electropherogram of IgG analysis using the PA 800 plus. Peaks: internal standard (10KD), light chain (LC), nonglycosylated heavy chain (NG), glycosylated heavy chain (HC).

Ordering Information

B37830	Biomek 4000 Workstation CE SDS-MW Configuration
A66528	PA 800 plus Pharmaceutical Analysis System
A66527	PA 800S plus Pharmaceutical Analysis System
390953	SDS-MW Analysis Kit
A10663	IgG Purity and Heterogeneity Assay
391734	IgG Control Standard

Results

Figure 3 shows a representative PA 800 plus electropherogram that illustrates the expected peaks for the internal standard, light chain (LC), nonglycosylated heavy chain (NG), and heavy chain (HC) peaks found in a typical reduced IgG sample. We analyzed the peak migration time, peak area, and corrected peak area (area/time) for multiple experiments of 24 replicate samples. Fully automated preparation including sample denaturation/reduction, dilution, and distribution into universal vials of IgG controls and buffers resulted in highly reproducible peak migration times with CVs <2% and corrected peak areas with CVs <3% using UV absorbance detection at 220 nm and demonstrated no cross contamination between samples.

Summary

Automation of buffer and sample preparation of recombinant proteins for molecular weight analysis by CE-SDS on the PA 800 plus provides significant advantages. High liquid transfer precision results in excellent assay reproducibility and low variability in peak area, thereby conferring high confidence in the data. By automating the complete process, significant walk-away time is gained and sample preparation throughput can be increased as scientists are freed from the bench. Finally, by automating up to 144 transfer steps, the likelihood of user error is reduced and sample tracking provides the assurance necessary for critical protein analysis.