

Automation of CE-SDS Sample Preparation for PA 800 Series IgG Purity/Heterogeneity Assays

Using a Biomek 4000 Automation Workstation

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Abstract

The SCIEX PA 800 Series was designed in collaboration with the pharmaceutical industry to maximize the advantages of capillary electrophoresis (CE) and has become the industry standard for CE-SDS analysis. However, CE-SDS sample preparation for the PA 800 Series is still a manual process, which can absorb significant operator time and remains a potential source of human error. Automation of the manual steps on the Beckman Coulter Biomek 4000 Workstation has resulted in reduced hands-on time and elimination of potential operator variability while delivering a robust assay.

In this application note, we demonstrate the automation of a CE-SDS workflow to prepare IgG protein samples on the Biomek 4000 Workstation. The fully automated denaturation and reduction of IgG controls resulted in highly reproducible peak migration times with CVs <2% and corrected peak areas with CVs <3% using UV absorbance at 220 nm. These results were accomplished using both β -mercaptoethanol (n=48) and TCEP (n=24) as reducing agents and demonstrated no cross contamination between samples.

Introduction

Although the PA 800 Series (Figure 1) replaces the manual process of pouring and running slab gels, sample preparation must still be performed manually. Manual sample preparation is time-consuming and remains a potential source of operator variation and human error. Automation of the sample preparation process ahead of CE-SDS analysis by the PA 800 Series has the potential to address these issues.

The sample preparation for CE-SDS analysis of MAbs on the PA 800 Series requires denaturation of the proteins by heating the samples in detergent, either in the presence or the absence of a reducing agent. Precise reagent and sample transfers are



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

essential for accurate protein quantification and robustness of mixing and heating are essential for accurate migration times. To run the prepared samples, the PA 800 Series 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 Series for analysis (Figure 2).

The Biomek 4000 Workstation (Figure 1) was used to automate the sample and buffer preparation for the IgG Purity and Heterogeneity Assay Kit from SCIEX. The automation method drives the denaturation/reduction of 1 to 24 samples (with optional normalization) as well as transfer of the PA 800 Series working buffers to buffer vials. Adapter plates were designed to hold the PA 800 Series buffer trays on the deck of the Biomek 4000 Workstation (Figure 1). Test samples consisted of a prepared IgG Control Standard (SCIEX) that contains a controlled quantity of the non-glycosylated heavy chain. Results with two different reducing agents showed excellent consistency in peak migration time and peak area across experiments with no cross contamination between samples.

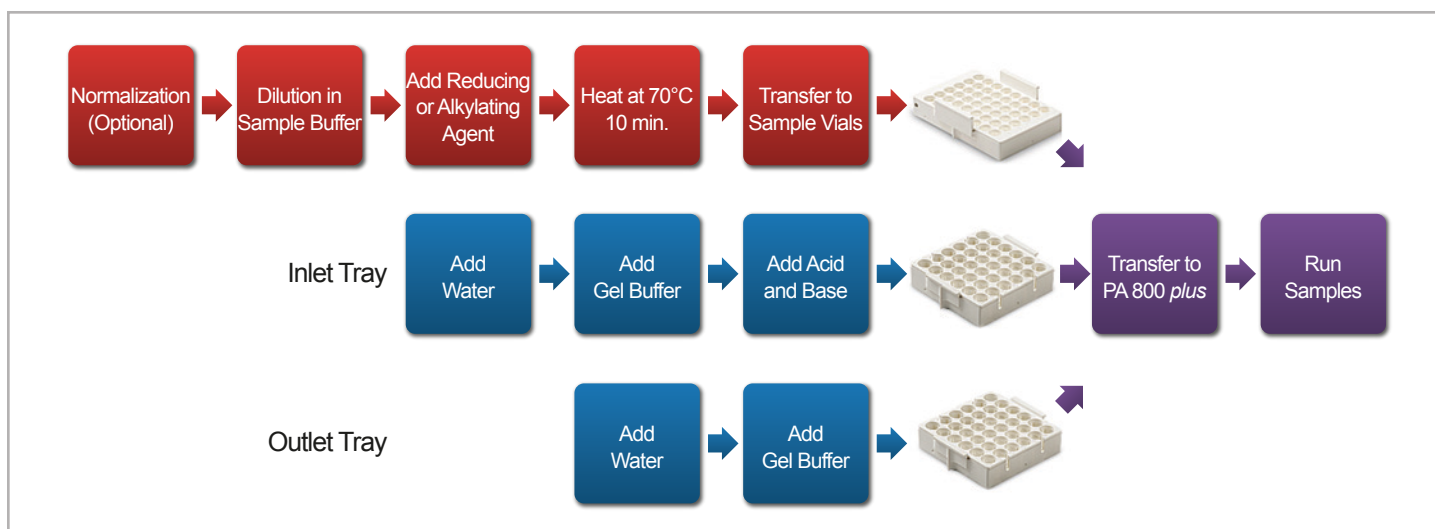


Figure 2. Process Workflow. The Biomek 4000 Workstation is used to automate the preparation of samples (red) and buffer trays (blue). The user transfers the sample tray and buffer trays to the PA 800 Series and runs the samples (purple).

Materials and Methods

IgG control samples (SCIEX) were prepared using the IgG Purity and Heterogeneity Assay Kit (SCIEX). The SDS-MW Kit (SCIEX) is also compatible with this method. Reducing agents used were 2-mercaptoethanol (β ME, Sigma Aldrich) and Tris(2-carboxyethyl) phosphine (TCEP, Sigma Aldrich). The Biomek 4000 Workstation was placed in a fume hood for the sample preparation to mitigate the inhalation hazard of β ME.

The automation method features a user interface that allows users to set reagent volumes and then select sample locations using a run-time pattern (Figure 3). If normalization is required, a .csv file (i.e. Microsoft Excel) that contains sample volume and location information is used. A reagent calculator indicates the

necessary total volumes of each reagent and the locations in the inlet/outlet trays that require buffer vials (Figure 3). The Biomek 4000 Workstation combines up to 24 samples with sample buffer, internal standard (optional) and either reducing agent or alkylating agent in a PCR plate. A typical deck layout is shown in Figure 4. This plate is then heated to 70° C for 10 minutes on an integrated shaking Peltier device. Inlet and outlet buffer trays are held on the deck of the instrument by an adapter plate (Figure 1) and buffer vials are filled with kit buffers (water, gel buffer, acid, and base). Denatured samples are transferred to sample vials for analysis on the PA 800 Series.

All samples were run on a PA 800 Series. All separations were performed using 50 μ m ID bare fused silica capillaries with a 30.2 cm total length and 20.0 cm effective length from the sample

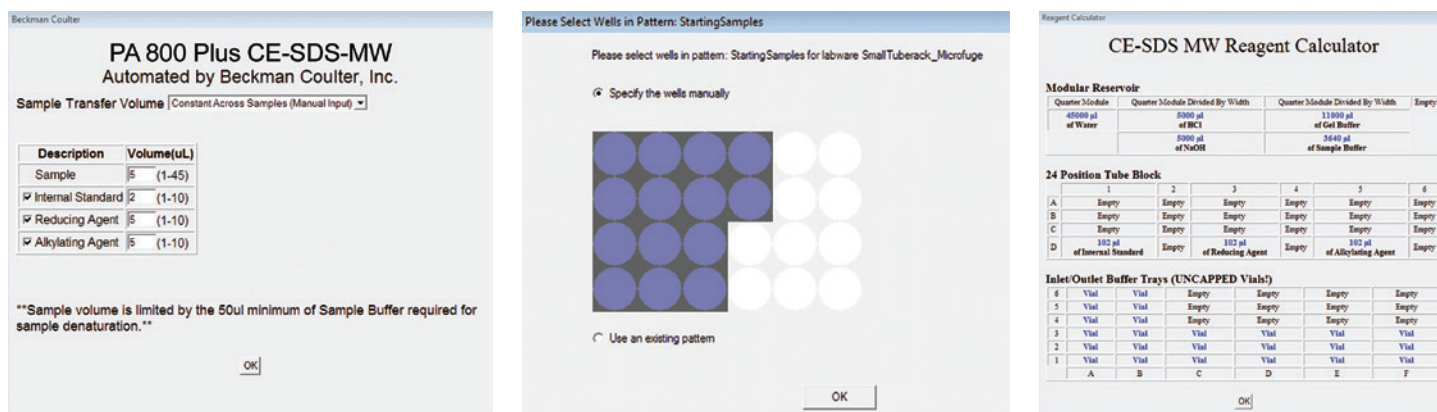


Figure 3. User Interface Screenshots. A) The user interface allows the selection of reagent and sample volumes and (B) sample locations using either a run-time pattern or a worklist file. C) The reagent calculator shows the location and total reagent volumes required, as well as the buffer tray locations that require buffer vials.

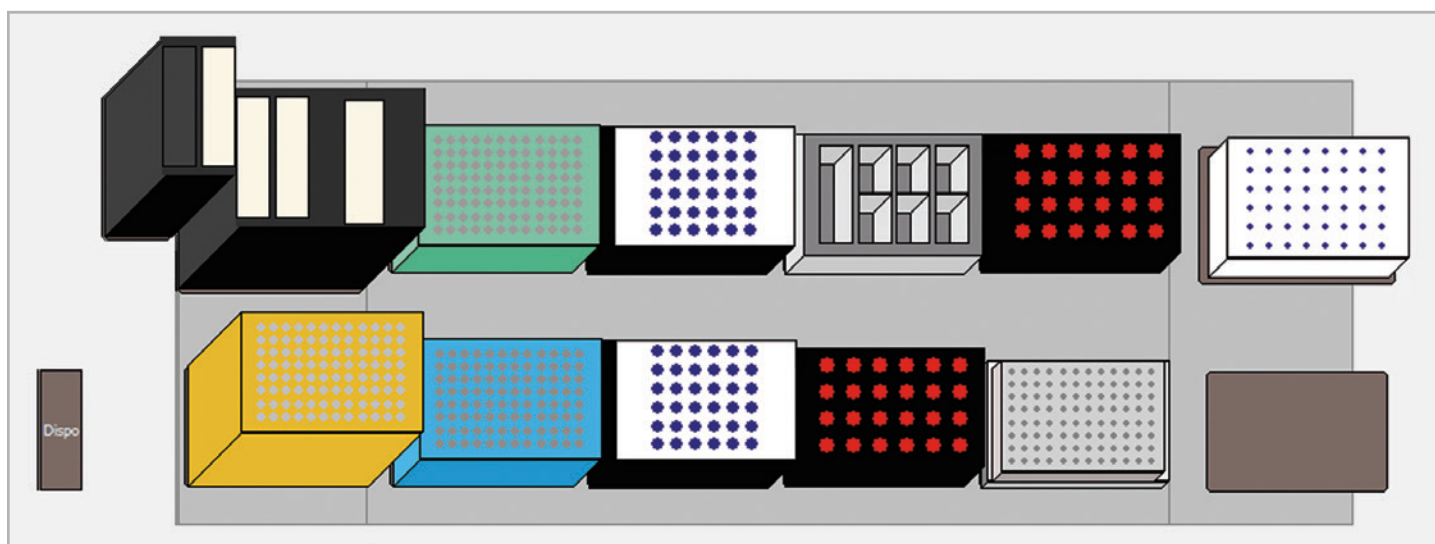


Figure 4. Biomek 4000 Workstation's Deck Layout. This software screenshot represents a typical deck layout for the IgG Purity and Heterogeneity Assay workflow. This setup includes the pipetting tools and gripper (upper left), tips (1000µL – yellow, 200µL – green, 20µL – blue), PA 800 Series inlet and outlet buffer trays, a modular reservoir for large volume reagents, tube racks for samples and small volume reagents, and the PA 800 Series sample vial tray. Samples are heated in a PCR plate ("Normalized Samples") on the integrated shaking Peltier device ("SPelt96_1").

introduction inlet to the detector window. All runs were performed using the SCIEX PA 800 Series IgG Purity/Heterogeneity Assay standard protocol (Application Guide A51967AD).

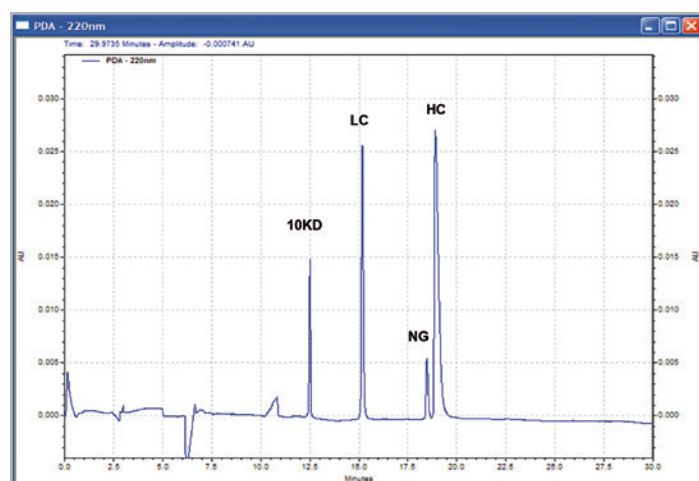


Figure 5. Electropherogram of IgG analysis using the PA 800 Series. Peaks: internal standard (10KD), light chain (LC), nonglycosylated heavy chain (NG), glycosylated heavy chain (HC).

Results and Discussion

Precise and Reproducible IgG Analysis

Figure 5 shows a representative PA 800 Series 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. The robustness of the combined automated sample preparation and PA 800 Series analysis is demonstrated by the low coefficient of variation (% CV) for these parameters. The %CV in migration time was <2%, while peak area and corrected peak area %CV were <3% (Table 1 and data not shown). In addition, the values for mobility, resolution, and relative peak areas all surpassed IgG control specification limits (i.e. mobility coefficient of variation <1%, data not shown).

To further characterize the automated method, we processed alternating samples and blanks and determined that there was no cross-contamination between wells (data not shown). We also processed samples with 100 mM TCEP as an alternative reducing agent due to its substantially lower toxicity than β-mercaptoethanol. Table 1 shows that denaturation and reduction of IgG in the presence of TCEP gives equivalent peak migration and area as when reduced with βME. The 24 samples were processed in this fashion and PA 800 Series buffers were dispensed to vials in a total of 90 minutes, reducing hands-on preparation time by roughly this amount.

A) β ME – Biomek Run #1 (n=24)**Migration Time**

	10 kd	LC	NG	HC
Mean (Min.)	12.86	15.61	19.00	19.44
Stdev (Min.)	0.10	0.12	0.16	0.16
% CV	0.80	0.79	0.83	0.82

B) β ME – Biomek Run #2 (n=24)**Migration Time**

	10 kd	LC	NG	HC
Mean (Min.)	12.54	15.22	18.52	18.95
Stdev (Min.)	0.12	0.16	0.21	0.22
% CV	0.97	1.06	1.13	1.14

C) TCEP – Biomek Run #3 (n=24)**Migration Time**

	10 kd	LC	NG	HC
Mean (Min.)	12.58	15.26	18.57	18.98
Stdev (Min.)	0.14	0.18	0.23	0.25
% CV	1.15	1.21	1.24	1.32

Corrected Peak Area

	LC	NG	HC
Mean	11284.15	2043.03	20532.93
Stdev	183.35	42.13	441.77
% CV	1.62	2.06	2.15

Corrected Peak Area

	LC	NG	HC
Mean	11688.91	2189.35	21467.08
Stdev	95.76	50.39	498.02
% CV	0.82	2.30	2.32

Corrected Peak Area

	LC	NG	HC
Mean	11923.49	2297.47	21582.15
Stdev	331.39	53.02	515.93
% CV	2.78	2.31	2.39

Table 1. IgG migration times and corrected peak areas following automated processing with either β ME (A, B) or TCEP (C) as the reducing agent. The mean, standard deviation, and coefficient of variation values from 24 replicate samples are shown. High reproducibility was seen in both migration times (CV <2%) and corrected peak areas (CV <3%).

Conclusions

Automation of buffer and sample preparation of recombinant proteins for molecular weight analysis by CE-SDS on the PA 800 Series 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.

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