Rapid and Accurate Protein Sizing, Quantitation, and Analysis Using the Experion™ Automated Electrophoresis System and the Experion Pro260 Analysis Kit

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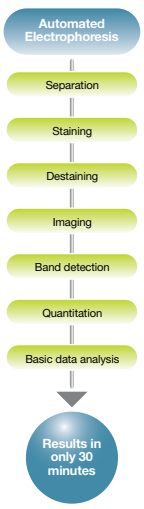
Introduction

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) is commonly used to determine the molecular weight (MW), concentration, and relative purity of a target protein. This method involves multiple steps, including electrophoresis, staining, destaining, imaging, and analysis; it usually takes at least 2–3 hr to complete this multistep process.

The Experion automated electrophoresis system offers an innovative method for performing electrophoresis by providing a compact microfluidics-based platform for rapid and automated analysis of protein samples. The Experion system integrates separation, detection, and data analysis, and displays the results in three formats in real time (Figure 1). The Experion Pro260 analysis kit is used for separation and analysis of 10–260 kD proteins, and requires only 30 min to complete the analysis of 10 samples. The Experion system also provides information not readily delivered by SDS-PAGE, such as the relative concentration and purity of a sample. Here we demonstrate that the performance of the Experion Pro260 analysis kit in the areas of separation range, sensitivity, sizing accuracy and reproducibility, quantitation reproducibility, and resolution is comparable to or superior to traditional SDS-PAGE (4–20% Tris-HCl gels).



Steps in automated analysis.

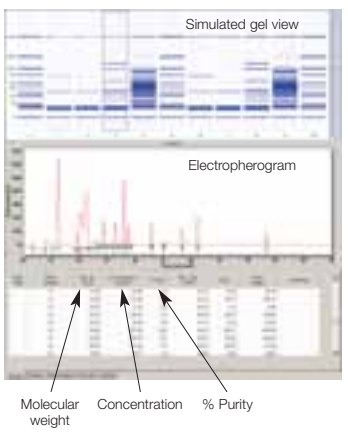


Fig. 1. The Experion system allows rapid separations using small sample volumes. Results are displayed in a simulated gel view, electropherogram, and Results table.

Methods

A lyophilized *E. coli* lysate at 2 mg/ml, bovine serum albumin (BSA) at 200 µg/ml, a 2.5–2,000 ng/µl carbonic anhydrase (CA) dilution series, and a blend of nine pure proteins (0.1-0.4 mg/ml per protein) were prepared in 1x phosphate-buffered saline (PBS). Samples for Experion Pro260 analysis were prepared by mixing 2 µl sample buffer containing β-mercaptoethanol with 4 µl sample, and loaded onto chips according to the protocol provided with the Pro260 analysis kit manual.

SDS-PAGE was performed using Criterion™ Tris-HCl 4–20% precast gels. Samples were prepared by combining 4 µl sample with 4 µl 2x Laemmli sample buffer containing β-mercaptoethanol. Gels were stained with Bio-Safe™ Coomassie stain, imaged on a GS-800™ densitometer, and analyzed with Quantity One® 1-D analysis software.

Results

Sizing range: Pro260 analysis and SDS-PAGE on 4–20% gradient gels display equivalent separation characteristics. Both produce a pattern of well-resolved 10–260 kD bands for the proteins in the Pro260 ladder (Figure 2A and B, far left lane on both images).

Sensitivity: The sensitivity of detection with the Pro260 analysis kit is equivalent to, or greater than, that achieved with a colloidal Coomassie Blue-stained SDS-PAGE gel (Figure 2). A 2.5–2,000 ng/µl dilution series of CA was analyzed, and in the simulated gel view produced by the Experion system (Figure 2A), the band associated with the most dilute sample (2.5 ng/µl, 10 ng total protein) was automatically identified by Experion software. In fact, the electropherogram shows that this dilute sample generated a peak with a mean signal-to-noise ratio of ≥20 (Figure 2C).

In contrast, the band associated with the same sample went undetected by the 1-D gel analysis software when separated in the gel (Figure 2B).

Protein sizing: Sizing data generated by the Experion system for a blend of nine 14–116 kD proteins were more accurate and more reproducible than those generated by Coomassie Blue-stained and imaged SDS-PAGE gels (Table 1).

Protein quantitation: The Experion system and SDS-PAGE analysis produced comparably reproducible quantitation results (Table 2). Only the Experion system, however, automatically determined the relative concentration of each protein detected.

Resolution: The Experion system provides resolution equivalent to or greater than that achievable with a 4–20% Tris-HCl gel (Figure 3 and Table 3).

- The numbers of distinct protein peaks or bands detected by each method in an *E. coli* lysate were comparable (Figure 3). Pro260 analysis revealed 35 peaks, and SDS-PAGE, 33 bands.
- Samples containing a pair of proteins with known MW differing by 1–13% were analyzed. In some cases, the chip separation showed greater resolution than the gel (Table 3, pairs C and F).

Table 1. Comparison of accuracy* and reproducibility of sizing of proteins in a protein mixture.** Proteins were separated using the Experion Pro260 analysis kit (n = 25) or SDS-PAGE (n = 25). MW values shown are mean ± SD.

		Experion			SDS-PAGE		
Protein	Expected MW (kD)	MW (kD)	Accuracy	Reproducibility	MW (kD)	Accuracy	Reproducibility
Lysozyme	14.3	14.23 ± 0.12	–0.49%	0.82%	12.10 ± 0.42	–15.38%	3.47%
β-Lactoglobulin	18.4	18.82 ± 0.13	2.26%	0.69%	14.70 ± 0.50	–20.11%	3.40%
Triosephosphate isomerase	26.6	26.10 ± 0.27	–1.86%	1.05%	24.00 ± 0.69	–9.77%	2.88%
Lactate dehydrogenase	36.5	33.37 ± 0.29	–8.56%	0.86%	32.20 ± 1.00	–11.78%	3.11%
Ovalbumin	45	44.43 ± 0.34	–1.26%	0.77%	42.30 ± 1.60	–6.00%	3.78%
Glutamate dehydrogenase	55	56.32 ± 0.47	2.39%	0.84%	51.40 ± 1.37	–6.55%	2.67%
Bovine serum albumin	66	71.60 ± 0.70	8.49%	0.97%	66.70 ± 1.78	1.06%	2.67%
Phosphorylase b	97	95.44 ± 0.67	–1.61%	0.71%	96.20 ± 2.75	–0.82%	2.86%
β-Galactosidase	116	123.00 ± 0.55	6.02%	0.45%	110.40 ± 3.06	–4.83%	2.77%

* Calculated as % difference relative to expected.
** Calculated as % CV.

Table 2. Comparison of reproducibility* of protein quantitation. Proteins were separated using the Experion Pro260 analysis kit or SDS-PAGE. The 260 kD ladder protein was not included in these data because it served as the internal standard in all wells.

	Experion			SDS-PAGE		
Protein Sample	# of Wells	Measured Conc. (ng/µl)	Reproducibility	# of Lanes	Band Density	Reproducibility
BSA	29	109.0 ± 4.70	4.3%	12	1.11 ± 0.09	8.41%
CA	30	161.0 ± 17.00	11.0%	12	0.15 ± 0.05	31.37%
Pro260 ladder						
10 kD band	29	157.1 ± 21.0	13.0%	16	0.40 ± 0.03	7.44%
20 kD band	29	182.3 ± 19.0	11.0%	16	0.45 ± 0.02	3.74%
25 kD band	29	160.7 ± 26.0	16.0%	16	0.39 ± 0.03	7.03%
37 kD band	29	110.1 ± 9.0	8.0%	16	0.38 ± 0.03	6.63%
50 kD band	29	119.9 ± 12.0	10.0%	16	0.38 ± 0.03	6.74%
75 kD band	29	127.1 ± 11.0	9.0%	16	0.45 ± 0.03	7.29%
100 kD band	29	105.0 ± 5.0	5.0%	16	0.41 ± 0.03	8.45%
150 kD band	29	67.0 ± 2.0	3.0%	16	0.26 ± 0.01	5.34%

* Calculated as % CV.

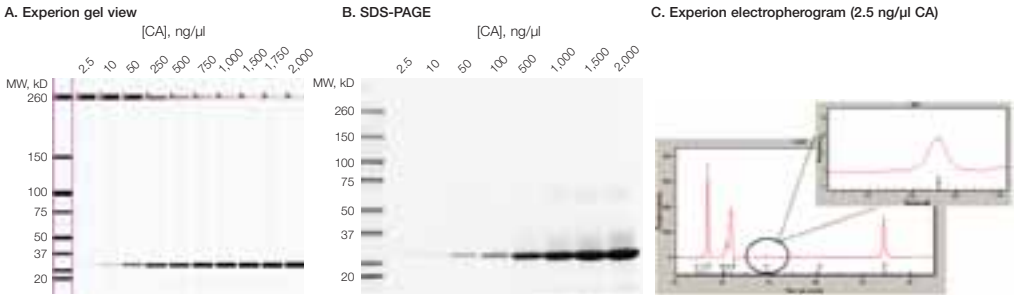


Fig. 2. Analysis of CA with the Experion system and by SDS-PAGE. CA samples were separated using the Experion Pro260 analysis kit (A) and SDS-PAGE (B); C, enlarged view of the 2.5 ng/µl CA peak (10 ng total protein) in an Experion electropherogram. Note the clear resolution of the CA peak from the baseline. Other peaks seen in the electropherogram include, left to right, the 1.2 kD lower marker, system peaks (generated by detergent-dye complexes), and the 260 kD upper marker.

Table 3. Comparison of resolution. Pairs of proteins were separated using the Experion Pro260 analysis kit or SDS-PAGE.

			Observed MW (kD)			Observed Difference	
	Protein Pair	Expected MW (kD)	Experion	SDS-PAGE	Expected Difference	Experion	SDS-PAGE
A	Pro260 ladder protein	100	100.11	97.2	3.09%	4.12%	5.54%
	Phosphorylase b	97	96.15	92.1			
B	Pro260 ladder protein	75	74.78	74.8	13.60%	4.15%	16.51%
	BSA	66	71.80	64.2			
C	Recombinant protein	53	52.50	50.8	6.0%	4.81%	0%
	Pro260 ladder protein	50	50.09	50.8			
D	Pro260 ladder protein	37	37.23	35.9	1.37%	10.84%	15.81%
	Lactate dehydrogenase	36.5	33.59	31.0			
E	Triosephosphate isomerase	26.5	26.50	22.7	6.40%	6.38%	3.18%
	Pro260 ladder protein	25	24.91	22.0			
F	β-Lactoglobulin	18.4	19.07	14.5	8.24%	12.37%	0%
	Myoglobin	17	16.97	14.5			

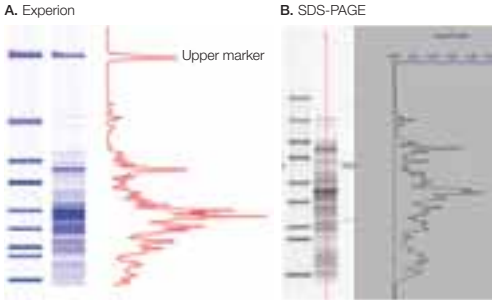


Fig. 3. Comparison of protein detection using an *E. coli* lysate. A, simulated gel view (left) and electropherogram (right) of separation with the Experion Pro260 analysis kit; the upper marker peak is indicated; **B**, gel image (left) and Quantity One software analysis (right) of separation by SDS-PAGE. A comparable number of peaks were identified using both separation methods.

Conclusions

The performance of the Experion Pro260 analysis kit in terms of separation range, sensitivity, accuracy and reproducibility of sizing, and reproducibility of quantitation and resolution is comparable to or even superior to traditional SDS-PAGE. Furthermore, automation of the multiple steps of gel electrophoresis through basic data analysis offers rapid results, minimal hands-on time, and greater ease of use.