Performance Comparison of the Experion™ Automated Electrophoresis System and SDS-PAGE for Protein Analysis

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Introduction

A fundamental task in many research laboratories is to analyze a protein sample to determine the size or molecular weight (MW), concentration, and relative purity of a target protein. Currently, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used to accomplish these tasks. While the availability of precast gels for SDS-PAGE has reduced the time required for gel preparation, the remaining steps of the process (electrophoresis, staining, destaining, imaging, and analysis) still demand significant amounts of hands-on time. In all, at least 2–3 hr are usually required to complete this multistep process.

The Experion automated electrophoresis system uses a combination of Caliper Life Sciences' innovative LabChip microfluidic separation technology and sensitive fluorescent sample detection to perform rapid and automated analysis of protein and RNA samples. By integrating separation, detection, and data analysis within a single platform, the Experion system eliminates many of the time-consuming manual steps described above. Protein analysis is performed using the Experion Pro260 analysis kit, which contains the reagents, microfluidic chips, and other supplies required for the separation and analysis of 10–260 kD proteins. A wide variety of protein samples, including cell lysates, chromatography column fractions, and purified proteins, can be analyzed with the Experion system and Pro260 analysis kit, and up to ten samples can be separated and analyzed in as little as 30 min. Besides offering significantly reduced time-to-results and hands-on time, the Experion Pro260 analysis kit offers other advantages over traditional gel electrophoresis, including lower sample and reagent volume requirements, specialized protein standards for accurate and reproducible sizing and quantitation, and decreased exposure to the hazardous chemicals typically associated with SDS-PAGE.

The Experion system also provides sample information not readily delivered by SDS-PAGE, such as the relative sample concentration and the purity of the sample. This tech note compares the performance of the Experion Pro260 analysis kit with traditional SDS-PAGE. Specifically, the sensitivity of detection, the reproducibility and accuracy of protein sizing, the resolution, and the reproducibility of quantitation by both methods were analyzed.

Methods

Protein Samples

Purified *E. coli* β-galactosidase, rabbit muscle phosphorylase b, bovine liver glutamate dehydrogenase, chicken egg ovalbumin, rabbit muscle lactate dehydrogenase, bovine milk β-lactoglobulin, chicken egg white lysozyme, equine heart myoglobin, and bovine erythrocyte carbonic anhydrase (CA) were purchased from Sigma-Aldrich, Inc. Bovine serum albumin (BSA) was purchased from the National Institute of Standards and Technology (NIST). Rabbit muscle triosephosphate isomerase was purchased from Boehringer Mannheim. A lyophilized *E. coli* lysate (Bio-Rad) and a proprietary 53 kD recombinant protein were also used.

All protein samples and mixtures were prepared in 1x phosphate-buffered saline (PBS). The lyophilized *E. coli* lysate was rehydrated to a final concentration of 2 mg/ml, BSA was prepared at a concentration of 200 µg/ml, and a 2.5–2,000 ng/µl CA dilution series was also used. For evaluations of protein sizing and quantitation performance, a blend of nine proteins (β-galactosidase, phosphorylase b, BSA, glutamate dehydrogenase, ovalbumin, lactate dehydrogenase, triosephosphate isomerase, β-lactoglobulin, lysozyme) was used; each protein was included at a final concentration of 0.4 mg/ml, except lactate dehydrogenase, which was included at 0.1 mg/ml. For protein pair separations, equivalent volumes of each protein preparation were combined prior to separation.

Experion Pro260 Analysis

Experion Pro260 analysis kits include the Experion Pro260 protein ladder, sample buffer, gel solution, fluorescent stain, spin filters, and microfluidic chips. Each chip holds up to ten samples; these were prepared by mixing 2 µl Pro260 sample buffer containing 3.2% β-mercaptoethanol with 4 µl sample, heating for 5 min at 95°C, and then diluting with 84 µl proteomic grade water. Samples were loaded onto chips according to the protocol provided in the Pro260 analysis kit instruction manual. At least five chips (number of wells ≥25) were run for each type of performance comparison.
For sensitivity studies, the signal-to-noise ratio was calculated as the ratio of the peak height to the standard deviation of the noise observed between 60 and 65 sec multiplied by 100.

SDS-PAGE

SDS-PAGE was performed using Criterion™ Tris-HCl 4–20% linear gradient precast gels and the Criterion cell. A 4–20% linear gradient gel was used because it yields separations most similar to those observed with the Experion Pro260 chip. Samples were prepared by combining 4 µl sample with 4 µl 2x Laemmli sample buffer containing 5% β-mercaptoethanol and heating at 95°C for 5 min before loading onto the gel. Electrophoresis was performed at a constant 200 V for 55 min. Gels were stained for 1 hr with Bio-Safe™ Coomassie stain, destained in water overnight, imaged on a GS-800™ densitometer, and analyzed with Quantity One® 1-D analysis software. At least four gels, with at least three replicates per gel (n ≥12), were run for each comparison.

Results and Discussion

Several aspects of protein separation, detection, and analysis were evaluated and compared between the microfluidics-based Experion system and traditional SDS-PAGE: sizing range, sensitivity of detection, linear dynamic range, accuracy and reproducibility of protein sizing, reproducibility of protein quantitation, and resolution.

Sizing Range

Using the Pro260 ladder as sample, the sizing range of the Pro260 analysis was compared to that of conventional SDS-PAGE. Both systems produced a pattern of well-resolved 10–260 kD bands, demonstrating that the Pro260 analysis and SDS-PAGE on 4–20% gradient gels have similar separation characteristics for the proteins in this ladder (Figure 1A and B, far left lane on both images, 10 kD band not shown).

Sensitivity and Linear Dynamic Range

To evaluate sensitivity and linear dynamic range, a 2.5–2,000 ng/µl dilution series of CA was prepared, and 4 µl samples at each concentration were analyzed using Pro260 chips or Tris-HCl gels.

In the simulated gel view produced by the Experion system (Figure 1A), 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 for this sample shows that the protein generated a peak with a mean signal-to-noise ratio of ≥20 (Figure 1C). In contrast, the band associated with the same sample went undetected by the 1-D gel analysis software when separated in the gel (Figure 1B). These results indicate that 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.

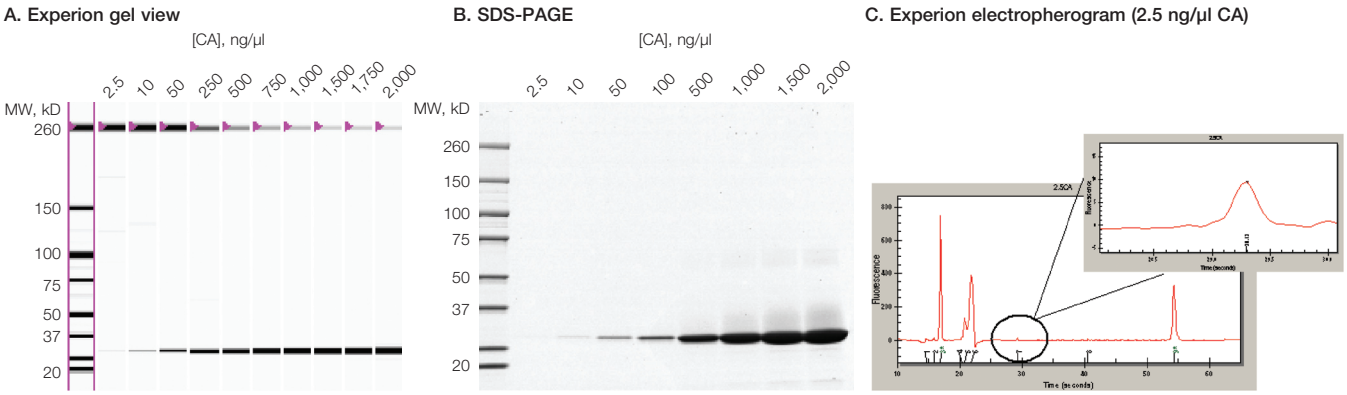

Fig. 1. Analysis of CA with the Experion system and by SDS-PAGE. Samples of CA at the indicated concentrations were separated using the Experion Pro260 analysis kit (A) and SDS-PAGE on 4–20% Tris-HCl gels (B); C, enlarged view of the peak generated by 2.5 ng/µl CA (10 ng total protein) in an Experion electropherogram. Note that the CA peak was clearly resolved from the baseline in the electropherogram. Other peaks seen in the electropherogram include, from left to right, the 1.2 kD lower marker, system peaks (generated by detergent-dye complexes), and the 260 kD upper marker.

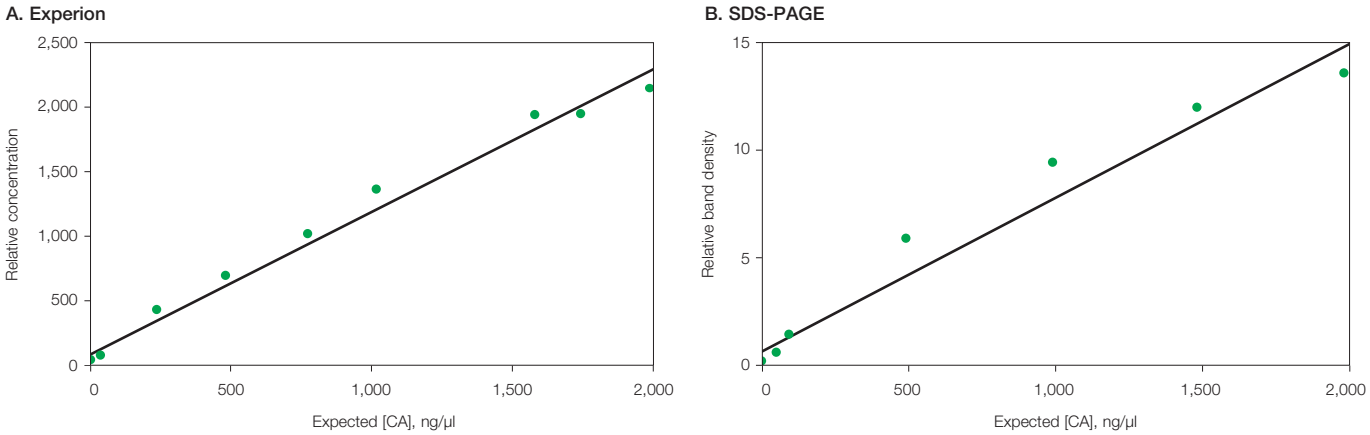

Fig. 2. Comparison of linear dynamic range of protein separation. Samples of CA at the concentrations indicated were separated with the Experion Pro260 analysis kit or by SDS-PAGE. The relative concentration or band density obtained from the Pro260 (A) and SDS-PAGE (B) analysis was plotted as a function of concentration. Representative graphs are shown. The r^2 values for a linear fit of the data sets were 0.9887 (A) and 0.9626 (B).

Any discussion of sensitivity must take into account the buffer composition, as the sensitivity of the Pro260 analysis is affected by the conductivity of the sample. Proteins are injected from the sample well into the separation channel of the Pro260 chip electrokinetically. Therefore, sample buffers with high ionic strength may cause less protein to be injected, thereby lowering the apparent sensitivity of the system. Conversely, salt concentrations below that of 1x PBS (150 mM NaCl), which is the buffer used here, may cause the injection of more protein than expected and an increased sensitivity. The inclusion of the upper marker within each sample, however, minimizes the potential impact of such salt effects on quantitation by the Experion system. Though SDS-PAGE is generally not influenced as greatly by changing salt concentrations, the sensitivity of this technique is affected by the width of the lane in which the sample is loaded. Additionally, the detection sensitivity of both systems can be affected by variations in protein staining and destaining, and by differences in peak or band width and shape.

The separation data were also plotted to evaluate the linear dynamic range for each protein separation platform. Representative plots of relative protein concentration (determined by Experion software) or relative band density (determined by Quantity One software) versus expected CA concentration are shown in Figure 2. The Pro260 data points were linear across the entire concentration range, and the coefficient of determination, r^2, varied from 0.97 to 0.99 (n = 5 chips). In contrast, r^2 ranged from 0.96 to 0.97 (n = 4 gels) for the 4–20% Tris-HCl gels. To achieve a linear fit to the gel data that was comparable to that determined by Experion software data analysis ($r^2 \approx 0.98$), it was necessary to reduce the CA concentration range examined to 10–1,000 ng/µl (not shown).

Accuracy and Reproducibility of Protein Sizing

The Experion system and SDS-PAGE workflows use similar approaches for MW determination (sizing); both rely on a calibration curve based on the migration of proteins of known MW (a protein ladder). Experion software automatically generates a calibration curve based on the migration time and

Table 1. Comparison of accuracy and reproducibility of sizing of proteins in the Pro260 ladder. The Experion Pro260 ladder was separated using the Experion Pro260 analysis kit (n = 29) or SDS-PAGE (n = 12). MW values shown are mean ± SD. The 260 kD ladder protein was not included in these data because it served as the internal standard in all wells.

	Experion			SDS-PAGE		
Expected MW, kD	MW, kD	Accuracy*, %	Reproducibility**, %	MW, kD	Accuracy*, %	Reproducibility**, %
10	9.96 ± 0.05	–0.37	0.47	10.00 ± 0.48	0.42	4.74
20	20.10 ± 0.19	0.44	0.93	18.80 ± 0.81	–6.08	4.29
25	25.10 ± 0.28	0.33	1.10	23.10 ± 0.91	–7.70	3.94
37	37.10 ± 0.36	0.39	0.98	36.30 ± 1.35	–1.96	3.71
50	50.20 ± 0.51	0.45	1.00	51.00 ± 1.89	2.00	3.71
75	75.50 ± 0.72	0.65	0.95	77.40 ± 2.85	3.14	3.69
100	100.40 ± 0.68	0.35	0.68	101.90 ± 3.74	1.90	3.67
150	150.40 ± 0.74	0.28	0.49	149.10 ± 5.32	–0.59	3.57

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

Table 2. 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.

known MW of each protein in the Pro260 ladder. The migration times of each protein in the sample wells are normalized to the ladder using internal upper and lower markers present in each sample and ladder well. The MW calibration curve is then used by Experion software to determine the size of each sample protein. For SDS-PAGE gels, relative migration (R_f) values for each protein band present in the ladder are used to derive a standard curve against which protein size determinations are made. In these studies, R_f values were exported from Quantity One software into an Excel spreadsheet, and a standard curve was derived for each of the ladder lanes on each gel by plotting the log (MW) versus R_f. The resulting equation was used to calculate the MW for each protein in the mixture being investigated. On both platforms, the Pro260 ladder was used as the standard against which size determinations were made.

To determine the accuracy and reproducibility of protein sizing, various mixtures of proteins were separated on both platforms. The sizes of the proteins in each mix were determined and then compared to their expected MW. Accuracy was defined as the percent difference between the calculated and expected protein size, with values close to zero signifying parity between the estimated size and the known size, and a negative or positive value indicating an underestimation or overestimation, respectively. Reproducibility was evaluated using the coefficient of variation, or CV ([standard deviation/mean] x 100), as a statistical measure; CV was expressed as a percentage, with small CV values indicating a small degree of variation in replicates and good reproducibility of the quantitative data.

Table 1 shows that when the Pro260 ladder was used, the Experion system delivered higher accuracy than SDS-PAGE on 4–20% Tris-HCl gels over the 10–150 kD range (deviation from expected size of –0.37 to 0.65% for the Experion system compared to –7.70 to 3.14% deviation for SDS-PAGE) as well as superior reproducibility (CV ≤1.10% for the Experion system compared to CV ≤4.74% for SDS-PAGE).

Another mixture, comprised of nine 14–116 kD proteins, was also separated by both systems and produced similar results. Table 2 shows that when sizing the protein mix against the Pro260 ladder, the Experion Pro260 analysis kit delivered more reproducible results (CV ≤1.05%) than did SDS-PAGE gels (CV ≤3.78%). In addition, the sizing estimates generated by the Pro260 analysis kit were more accurate, deviating from expected sizes by –8.56 to 8.49%, compared to the –20.11 to 1.06% deviation found with the SDS-PAGE gels (Table 2).

Table 3. 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	109.00 ± 4.70	4.30	12	1.11 ± 0.09	8.41
CA	30	161.00 ± 17.00	11.00	12	0.15 ± 0.05	31.37
Pro260 ladder						
10 kD band	29	157.10 ± 21.00	13.00	16	0.40 ± 0.03	7.44
20 kD band	29	182.30 ± 19.00	11.00	16	0.45 ± 0.02	3.74
25 kD band	29	160.70 ± 26.00	16.00	16	0.39 ± 0.03	7.03
37 kD band	29	110.10 ± 9.00	8.00	16	0.38 ± 0.03	6.63
50 kD band	29	119.90 ± 12.00	10.00	16	0.38 ± 0.03	6.74
75 kD band	29	127.10 ± 11.00	9.00	16	0.45 ± 0.03	7.29
100 kD band	29	105.00 ± 5.00	5.00	16	0.41 ± 0.03	8.45
150 kD band	29	67.00 ± 2.00	3.00	16	0.26 ± 0.01	5.34

* Calculated as % CV.

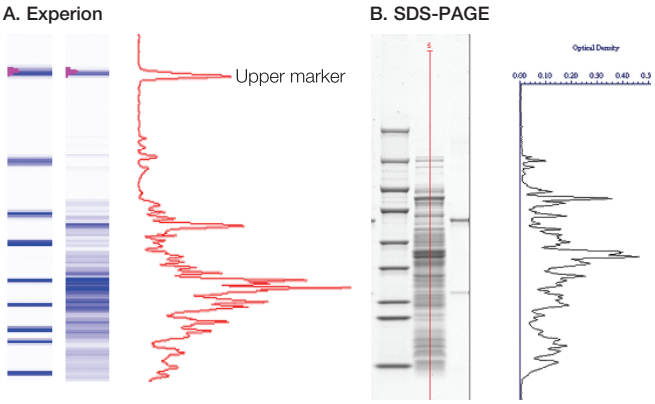

Fig. 3. Comparison of protein detection using an *E. coli* lysate. A, simulated gel view (left) and electropherogram (right) of separation with the Experion Pro260 analysis kit; the peak generated by the upper marker is indicated; **B**, gel image (left) and Quantity One software analysis (right) of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

The data in Table 2 also indicate that the least accurate sizing occurred for low MW proteins separated by 4–20% SDS-PAGE gels, while no size-dependent effect was obvious for the Pro260 analysis kit separations. A similar size dependence was not observed for the SDS-PAGE data in Table 1; this is likely because, in this case, the same Pro260 ladder proteins were used to create the standard curve against which sizing estimates were made. On the other hand, it is important to note that SDS-PAGE analysis offers the flexibility to select the gel percentage that is most accurate for sizing a particular MW range.

Reproducibility of Protein Quantitation

In addition to providing MW assignments, the Experion system automatically determines the relative concentration of each protein detected. To demonstrate the reproducibility of quantitation achieved with the Pro260 analysis and SDS-PAGE, three protein samples were separated on Pro260 chips and on 4–20% Tris-HCl gels: the Experion Pro260 ladder, 100 ng/µl CA, and 100 ng/µl BSA.

The Experion and SDS-PAGE analyses produced comparably reproducible quantitation results (Table 3). With the Pro260 analysis kit, the variation in the CV for quantitation was 3–16% for the proteins examined, and with SDS-PAGE, a variation of 3.7–31.4% was seen, with the largest variation likely caused by poor staining of CA.

Resolution

The Experion Pro260 analysis kit was designed to provide resolution equivalent to or greater than that achievable with a 4–20% Tris-HCl gel. To demonstrate this equivalence, a complex E. coli lysate was analyzed using both methods, and the numbers of distinct protein peaks or bands detected by each method were compared. Whereas the Pro260 analysis reproducibly separated and identified an average of 35 protein peaks using the default settings, an average of 33 peaks were detected for the gel-based separations using the band plot function of Quantity One software (noise filter setting = 4.0, shoulder sensitivity = 1.0) (Figure 3).

Resolution can also be described using a resolution value, R_s, which is defined by the mathematical equation $R_s = 1.17 \times (t_2 - t_1)/(w_1 + w_2)$, where t_1 = migration time of peak 1, t_2 = migration time of peak 2, w_1 = width of peak 1 at half height, and w_2 = width of peak 2 at half height. R_s describes how well a chromatographic or other separation system can resolve or separate two molecules. The equation assumes that the two molecules generate similarly shaped peaks. $R_s \geq 1.5$ means that the system can completely separate (baseline resolve) the two species such that the peak signal from the first peak returns to baseline before the signal of the second peak increases.

Based on repeated separations of the Pro260 ladder using the Experion system, calculations of R_s were made for similar proteins differing in MW by 10%. These R_s values increased with increasing MW, from $R_s = 1.2$ at 10 kD to $R_s = 2.0$ at 260 kD (data not shown), indicating that proteins greater than 25 kD (where $R_s = 1.5$) and differing by 10% in size can be baseline separated by the Pro260 assay.

To further explore the resolving capability of the Pro260 analysis kit versus conventional SDS-PAGE, several samples, each containing a pair of proteins with known MW differing by 1–13%, were analyzed. Although in many cases both methods resolved the protein pairs as distinct peaks or bands (Table 4 and Figure 4, panels A, B, D, and E), in some cases the chip separation showed greater resolution than the gel (Table 4 and Figure 4, panels C and F).

Besides showing that the Experion system separates proteins comparably to or better than precast gradient gels, this experiment also demonstrates that the effective resolution for both systems depends on other factors, such as the width of peaks and bands and variations in protein migration times. For example, BSA migrates fairly true to its theoretical MW on 4–20% SDS-PAGE gels, but migrates as an approximately 72 kD protein in the chip. The cause of these alterations in migration may be due in part to differences in the composition and pore structure of the separation matrix and, in the case of chip separations, the fact that staining occurs throughout separation, whereas staining is a postseparation event for SDS-PAGE analysis.

Table 4. Comparison of resolution.

Protein Pair		Expected MW, kD	Observed MW, kD		Expected Difference, %	Observed Difference	
			Experion	SDS-PAGE		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			

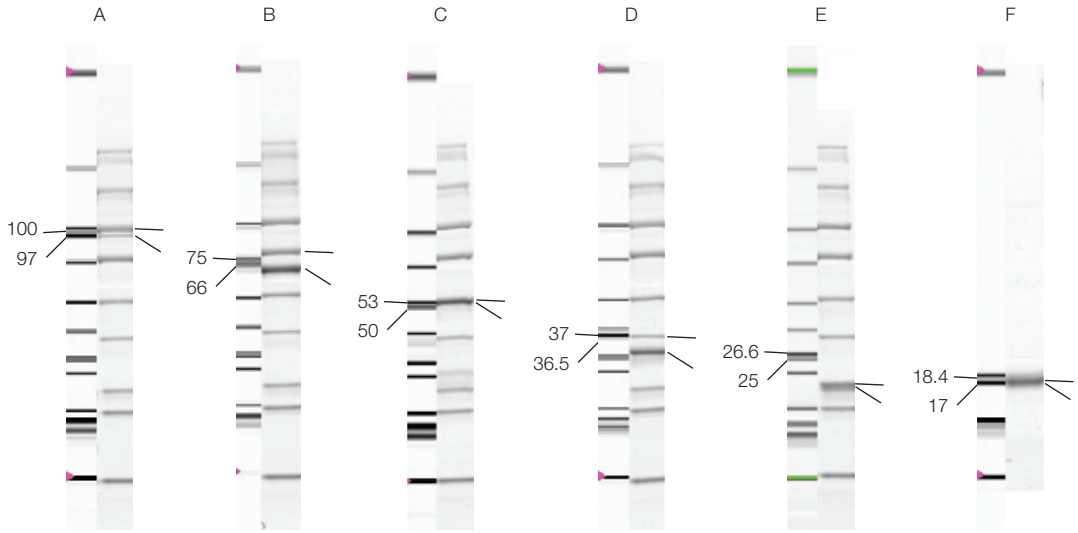

Fig. 4. Comparison of separation of pairs of proteins with the Experion Pro260 analysis kit and SDS-PAGE. For each protein pair listed in Table 4, the left panel shows the Experion simulated gel view, and the right panel, a scanned SDS-PAGE gel image.

Conclusions

The performance of the Experion Pro260 analysis kit in terms of separation range, sensitivity, linear dynamic range, resolution, accuracy and reproducibility of sizing, and reproducibility of quantitation is comparable to or even superior to traditional SDS-PAGE. Furthermore, the automation of the multiple steps of gel electrophoresis through basic data analysis offers rapid results, minimal hands-on time, and greater ease of use. The Experion system and Pro260 analysis kit support a wide variety of protein applications, including quality control, protein purity and stability analysis, protocol optimization, and recombinant protein expression evaluation by providing fast, well-resolved, and reproducible results.

Information in this tech note was current as of the date of writing (2005) and not necessarily the date this version (Rev B, 2010) was published.

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