Moving Beyond Gels

The Advantages of CE-SDS Over SDS-PAGE

Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) delivers the same essential separation as SDS-PAGE but with greater precision, quantitation, and automation. Unlike the labor-intensive gel method, CE-SDS streamlines protein analysis with higher reproducibility, eliminating variability from manual staining and band interpretation. Its superior resolution ensures more accurate molecular weight determination, while automated workflows reduce hands-on time and operator dependence[1,2]. With quantitative peak integration replacing qualitative band intensity assessments, CE-SDS offers a more reliable and reproducible approach for protein characterization, making it better suited for analytical applications in regulated environments. CE-SDS has already been widely tested and validated in the biopharmaceutical industry for the analysis of various biotherapeutics[3,4,5].

This application note presents a comparison of the results generated from SDS-PAGE and CE-SDS, with a focus on a monoclonal antibody and AAV sample. The **Maurice™ system** and **Turbo CE-SDS™ cartridge** were used for CE-SDS separation in this study, providing results in as little as 5.5 minutes per sample.

Phase	Sample Prep	Gel Setup	Load Sample	Run Gel	Staining and Destaining	Data Analysis
SDS-PAGE						
Duration	20 min	15 min	10 min	50 min	30 min - 4 hr	1 hr
Waste			1-2 Acrylamide Gels	30-50 mL	20-50 mL	

Phase	Sample Prep	Instrument Setup	Analyze Samples	Data Analysis
CE-SDS				
Duration	20 min	10 min	65 min	5-30 min
Waste		3 mL	5 mL	

Figure 1. A visual summary of the differences between SDS-PAGE and CE-SDS workflows for 12 samples of a typical monocolonal antibody. Significant savings on time and a reduction in waste generation are some of the many advantages of CE-SDS.

Materials and Methods

All materials used in this study are listed in **Table 1**, including the Maurice Turbo CE-SDS Size Application Kit that contains all the necessary reagents for CE-SDS analysis on the Maurice system.

TABLE // 01

Material	Vendor	Catalog #
Benlysta® (Belimumab)	GlaxoSmithKline	NA
AAV8-CMV-GFP & AAV9-CMV-GFP from HEK Cells	Virovek	NA
Maurice System	Bio-Techne	**090-158**
Maurice Turbo CE-SDS Cartridge		**PS-MC02-TS**, **PS-MC01-TS**
Maurice Turbo CE-SDS Size Application Kit		**PS-MAK01-TS**
Criterion™ Cell and PowerPac™ Basic Power Supply	Bio-Rad	1656019
10X Premixed Electrophoresis Buffer		1610732
10% Criterion TGX Stain-Free™ Protein Gel		5678033
2X Laemmli Sample buffer		1610737
Precision Plus Protein Kaleidoscope MW Standards		1610375
Iodoacetamide (IAM)	Millipore Sigma	16125
β-Mercaptoethanol (β-ME)		M-3148

Table 1. List of materials and reagents used in the study.

Method for Analysis with CE-SDS

Belimumab samples were prepared by diluting the stock solution (80 mg/mL) to 2 mg/mL in deionized water, followed by further dilution to a final concentration of 0.5 mg/mL using the Maurice 1X CE-SDS PLUS Sample Buffer. Maurice CE-SDS 25X Internal Standard (4%) was added to the samples, followed by the addition of either 5% (V/V) IAM (250 mM) for non-reduced analysis or 5% (V/V) β-ME (14.2 M) for reduced analysis. Samples were heated at 70°C for 10 minutes, cooled on ice for 5 minutes, and subjected to centrifugation. Finally, all samples were further diluted with an equal volume of deionized water. Samples were loaded onto the Maurice instrument, injected for 8 seconds at 3500 V, and separated at 4200 V for either 8 minutes (non-reduced) or 5.5 minutes (reduced). The data were analyzed with Compass for iCE software.

Both AAV8 and AAV9 samples were prepared using the same method. A 100 µL aliquot of AAV was mixed with 400 µL cold acetone, followed by incubation at -80°C for 1 hour. It should be noted that CE-SDS can analyze as little as 1 µL of AAV. Samples were centrifuged at 13,200 rpm for 10 minutes, and the supernatant was discarded. The resulting pellet was air-dried and resuspended in 100 µL of Maurice 1X CE-SDS Sample Buffer containing 4 µL of Maurice CE-SDS 25X Internal Standard (4%) and 5 µL of β-ME (14.2 M). Samples were heated at 70°C for 10 minutes, cooled on ice for 5 minutes, and centrifuged at 13,200 rpm for 5 minutes. The supernatant was collected, diluted with 400 µL of deionized water, and loaded into a 96-well plate (100 µL per well). Samples were loaded onto the Maurice instrument and injected for 8 seconds at 3500 V, followed by separation for 8 minutes at 4200 V. All data were analyzed with Compass for iCE software.

Method for Analysis with SDS-PAGE

Belimumab samples were prepared at a final concentration of 0.5 mg/mL by a 1:1 dilution with the 2X Laemmli Sample Buffer. Reduced samples were prepared with β-ME (5% V/V) while non-reduced samples were prepared without β-ME. A total of 20 µL of each sample was loaded on each lane. Gels were run under constant voltage at 200 V for approximately 50 minutes. Following electrophoresis, gels were scanned using an imaging system, and quantitative analysis was performed using AlphaView software.

Both AAV8 and AAV9 samples were prepared using the same method. 120 µL of each AAV sample was mixed with 480 µL ice-cold acetone and incubated at -80°C for 1 hour. The sample was centrifuged at 13,200 × g for 10 minutes at 4°C to pellet the proteins. The supernatant was removed and discarded, and the pellet was air-dried, followed by resuspension in 270 µL of 1X Laemmli buffer (reduced) prepared by combining 200 µL 2X Laemmli buffer, 10 µL β-ME, and 190 µL dH_2O. The sample was heated at 70°C for 10 minutes, cooled on ice for 3 minutes, and centrifuged at 13,200 × g for 10 minutes. 20 µL of each sample was loaded on each lane. A total of 4 lanes were used for AAV8 and 4 lanes for AAV9. The gel was run at a constant voltage of 200 V for ~50 minutes. Gels were scanned using an imager, and quantitative results were obtained using AlphaView software. Aliquots of 90 µL 1X sample were stored at -80°C until use.

Results

Belimumab

Figures 2A and **2B** are representative electropherograms of Belimumab, generated with the Turbo CE-SDS cartridge under non-reduced and reduced conditions, respectively. The peaks shown correspond to various bands that are typical of gel electrophoresis results. Minor peaks (1-3) are detected with CE-SDS but not with gels. Importantly, the Compass for iCE software features Lane View, a dynamic visualization tool that offers CE-SDS results in a gel-like representation (**Figure 3A**). The results shown from this view are consistent with those from SDS-PAGE, shown in **Figure 3B**.

FIGURE // 02

Typical size profiles of the monoclonal antibody Belimumab with the Maurice Turbo CE-SDS method

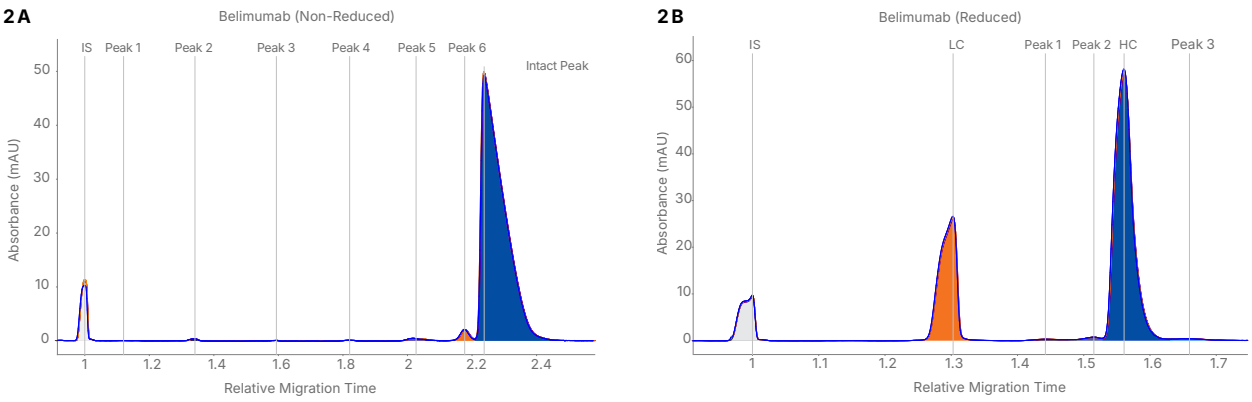
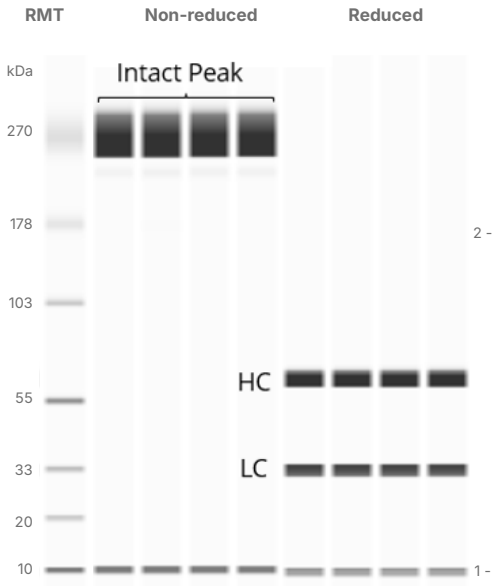


Figure 2. Typical size profiles of the monoclonal antibody Belimumab with the Maurice Turbo CE-SDS method. A. The electropherogram generated under non-reduced conditions shows the intact peak, with detection of other minor peak. B. The electropherogram generated under reduced conditions shows the well-resolved light chain (LC) and heavy chain (HC), along with other minor peaks. IS refers to the Internal Standard, which is supplied in the Maurice Turbo CE-SDS Size Application Kit.

FIGURE // 03

Lane analysis of the size profiles of Belimumab

3A



3B

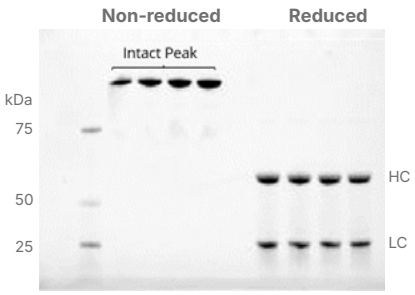


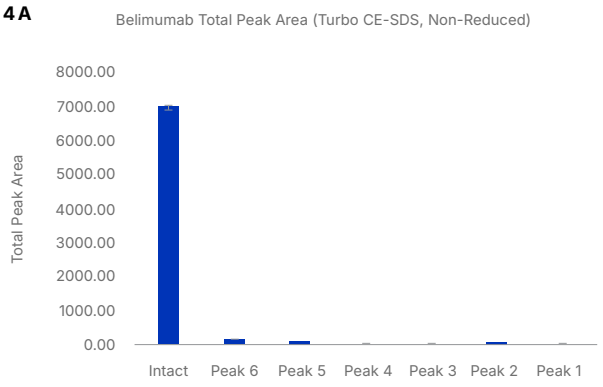
Figure 3. Lane analysis of the size profiles of Belimumab. A. The virtual gel image generated with the Lane View feature on Compass for iCE shows bands for the intact peak under non-reduced conditions, with a dominant intact band along with faint bands corresponding to minor species, and shows bands for HC and LC under reduced conditions. B. The SDS-PAGE gel run under non-reduced and reduced conditions shows similar bands to those from Turbo CE-SDS, indicating the comparability of both methods.

One of the many critical differences between both methods is sensitivity. While SDS-PAGE only detected the intact peak of non-reduced Belimumab, CE-SDS revealed the presence of minor peaks. While easier to see in the electropherogram, the faint bars seen through the Lane View tool correspond to those minor peaks. Because SDS-PAGE showed only the intact peak under non-reduced conditions, the total peak area was calculated, as shown in **Table 2. Figures 4A** and **4B** show bar graphs of the total peak area with Turbo CE-SDS and SDS-PAGE, respectively, under non-reduced conditions. The bar graph for the CE-SDS method has an acceptable error bar, which reflects the standard deviation. In contrast, the large error bar in **Figure 4B** indicates high variability between gels and between lanes, which are typical drawbacks of SDS-PAGE.

FIGURE // 04

Comparison of total peak area reproducibility between Maurice Turbo CE-SDS and SDS-PAGE

4A



4B

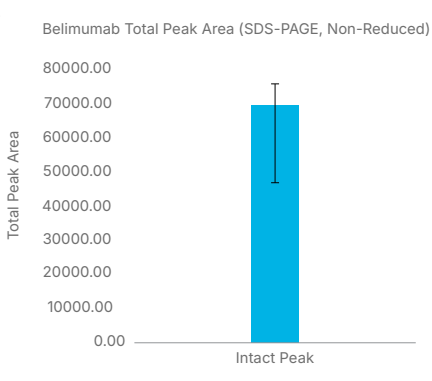


Figure 4. Comparison of total peak area reproducibility between Maurice Turbo CE-SDS and SDS-PAGE. A. The bar graph represents the total peak area obtained using the Turbo CE-SDS method, showing a small error bar that reflects low variability for 34 consecutive injections. B. The bar graph represents the total peak area obtained using SDS-PAGE, where the large error bar indicates high variability between gels and between lanes for a total of 3 gels with 4 lanes each.

TABLE // 02

Belimumab Total Peak Area of Intact Peak (Non-Reduced)

	Turbo CE-SDS (n=34 Injections)	SDS-PAGE (n=3 Gels, 12 Lanes Total; 4 Lanes/ Gel)
Average	6999.09	61254.33
Standard Deviation	72.80	14434.02
%RSD	1.04	23.56

Table 2. Summary of the total peak area of the intact peak for Belimumab. A stark difference in the %RSD values between both methods highlights the better reproducibility of the CE-SDS method.

Figures 5A and **5B** show bar graphs of the percent peak area (%PA) of the LC and HC detected with Turbo CE-SDS and SDS-PAGE, respectively. Additional minor peaks are shown for the Turbo CE-SDS, owing to the greater sensitivity of the method. The results are summarized in **Table 3**. %RSD values for Turbo CE-SDS are ≤0.44 for HC and LC, which starkly contrast to those for SDS-PAGE (%RSD ≤9.21).

FIGURE // 05

Comparison of Belimumab percent peak area reproducibility between Maurice Turbo CE-SDS and SDS-PAGE

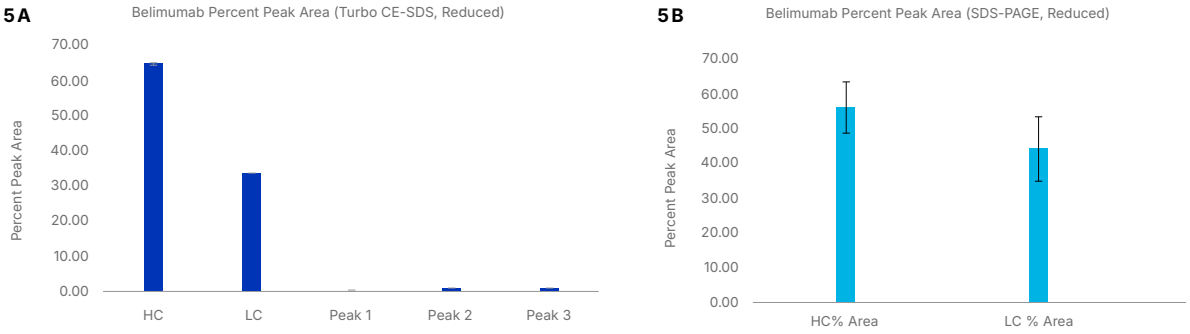


Figure 5. Comparison of Belimumab percent peak area reproducibility between Maurice Turbo CE-SDS and SDS-PAGE. A. The bar graph shows the percent peak area of each peak detected using the Turbo CE-SDS method, with a very small error bar that indicates high reproducibility between 34 injections. B. The bar graph represents the percent peak area for HC and LC using SDS-PAGE, and the larger error bar indicates higher variability between a total of 3 gels with 12 lanes each.

TABLE // 03

Belimumab Percent Peak Area (Reduced)

	Turbo CE-SDS (n=34 Injections)		SDS-PAGE (n=3 Gels, 4 Lanes Each)	
	HC	**LC**	**HC**	**LC**
Average	64.80	35.53	55.91	44.09
Standard Deviation	0.26	0.15	4.06	4.06
%RSD	**0.40**	**0.44**	**7.26**	**9.21**

Table 3. Summary of the %PA of Belimumab. %RSD values for the LC and HC from the CE-SDS method are significantly lower than those from SDS-PAGE, indicating a difference in method reproducibility.

AAV Samples

Figures 6A and **6B** are representative electropherograms of AAV8 and AAV9, respectively, where the viral capsid proteins (VP1, VP2, and VP3) are well-resolved with the Turbo CE-SDS method. Additionally, low molecular weight (LMW) species and other minor peaks are visible. A gel-like representation of CE-SDS results of AAV8 and AAV9 was obtained with the Lane View tool (**Figure 7A**), offering a direct visual comparison to traditional SDS-PAGE results (**Figure 7B**).

FIGURE // 06

Representative electropherograms of AAV serotypes with CE-SDS

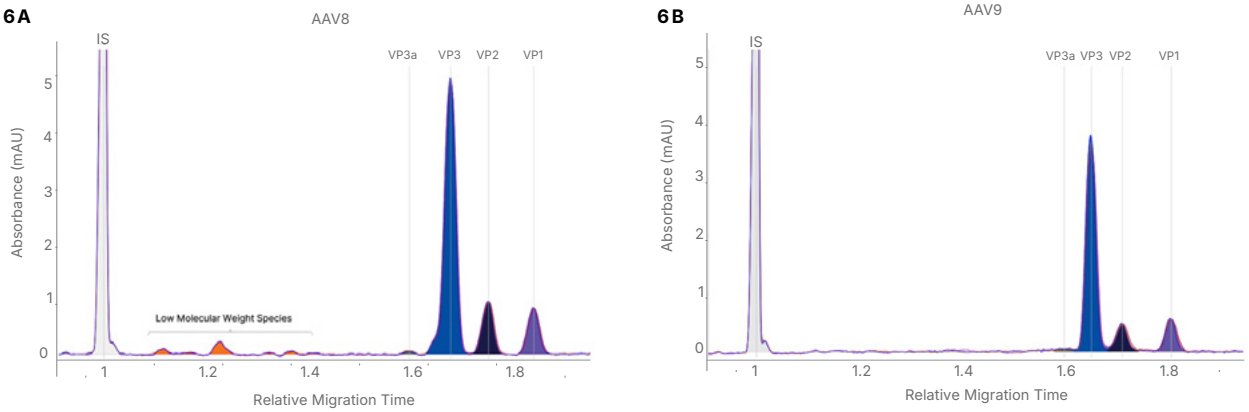


Figure 6. Representative electropherograms of AAV serotypes with CE-SDS. A. The electropherogram of AAV8 shows the three distinct viral proteins along with a minor modification, labeled as VP3a, and some LMW species. B. The electropherogram of AAV9 shows similar peaks to AAV8, but with fewer impurities.

FIGURE // 07

Lane analysis of AAV8 and AAV9 samples

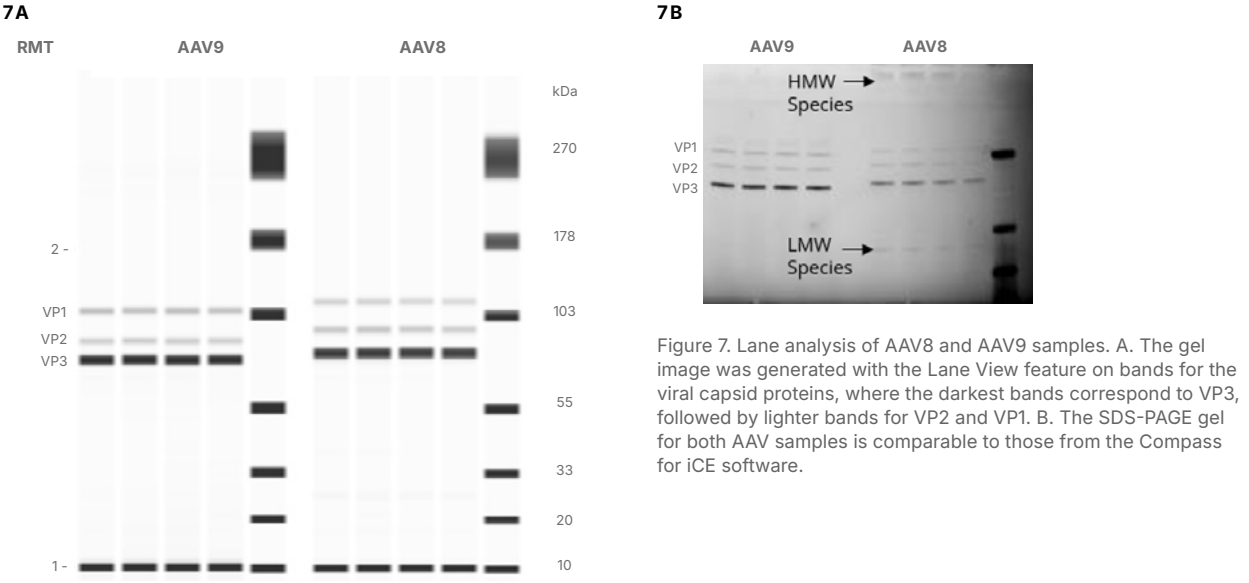


Figure 7. Lane analysis of AAV8 and AAV9 samples. A. The gel image was generated with the Lane View feature on bands for the viral capsid proteins, where the darkest bands correspond to VP3, followed by lighter bands for VP2 and VP1. B. The SDS-PAGE gel for both AAV samples is comparable to those from the Compass for iCE software.

Similar to the example of Belimumab in the previous section, the biggest difference between CE-SDS and SDS-PAGE is quantitative reproducibility. While SDS-PAGE detected VP bands, CE-SDS resolved them with higher sensitivity, allowing for a more precise determination of percent peak area (%PA). The difference in reproducibility between the two methods is highlighted in **Tables 4** and **5**, which provide quantitative results on VP3, VP2, and VP1 for both AAV8 and AAV9. In another study, CE-SDS on the Maurice system was used to analyze different Critical Quality Attributes (CQAs) and the capsid protein ratio of AAV samples[6].

TABLE // 04

AAV8 Percent Peak Area

	Turbo CE-SDS (n=35)					SDS-PAGE (n=3 Gels, 4 Lanes Each)				
	VP3	**VP2**	**VP1**	**VP3a**	**LMW Species**	**VP3**	**VP2**	**VP1**	**HMW Species**	**LMW Species**
Average	69.07	12.69	10.57	0.52	7.15	45.83	7.53	6.69	8.73	4.59
Standard Deviation	0.41	0.17	0.14	0.10	0.35	4.76	0.69	1.26	7.18	3.29
%RSD	**0.59**	**1.33**	**1.37**	19.04	4.82	**10.39**	**9.11**	**18.89**	82.22	71.80

Table 4. Summary of the %PA of AAV8. %RSD values for VP3, VP2, and VP1 %PA calculated for AAV8 with CE-SDS are low and well within the acceptable range, while those from the SDS-PAGE method are markedly higher, once again illustrating the difference in reproducibility between the two methods.

TABLE // 05

AAV9 Percent Peak Area

	Turbo CE-SDS (n=35)				SDS-PAGE (n=3 Gels, 4 Lanes Each)		
	VP3	**VP2**	**VP1**	**VP3a**	**VP3**	**VP2**	**VP1**
Average	75.35	11.34	11.90	1.39	77.55	9.87	12.58
Standard Deviation	0.73	0.56	0.29	0.22	0.91	0.50	0.86
%RSD	**0.97**	**4.92**	**2.44**	15.94	**1.18**	**5.05**	**6.82**

Table 5. Summary of the %PA of AAV9. %RSD values for VP3, VP2, and VP1 %PA calculated for AAV8 with CE-SDS are within the acceptable range. Results from SDS-PAGE analysis are also within the acceptable range in this case, but still higher than those from CE-SDS.

Conclusion

This study presented a comparison between Turbo CE-SDS and traditional SDS-PAGE methods and highlighted the advantages of CE-SDS in detecting subtle peaks and minor modifications with higher sensitivity and precision. The Compass for iCE software's Lane View feature provides a user-friendly interface for visualizing results akin to gel electrophoresis. Additionally, CE-SDS demonstrates superior quantitative reproducibility, as shown by the smaller error bars and enhanced accuracy in determining percent peak areas (%PA). These findings underscore the potential of CE-SDS as a versatile and reliable tool for characterizing proteins, particularly in applications requiring detailed resolution and quantification. By offering improved sensitivity, reproducibility, better quantitation, and ease of analysis, CE-SDS emerges as a valuable technique for researchers seeking precise protein profiling and characterization.

Learn more about CE-SDS

Scan the QR Code or Visit:

bio-techne.com/ce-sds

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6. Application Note – **Characterize Your Viral Vectors from Discovery to GMP Release with the Maurice System**