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CE-SDS Protein Kit

Introduction

Molecular weight determination of proteins using traditional SDS-PAGE is a time consuming, manual, multi-step process with limited quantitative accuracy. Capillary electrophoresis provides a rapid, alternative method to separate, quantify, and determine the molecular weight of proteins from 14–200 kDa. Impurities such as protein variants can be detected and quantified at levels below 1% of total protein content.

Bio-Rad's new CE-SDS Protein Kit utilizes a polymer solution to create a dynamic sieving effect within a capillary that is analogous to the crosslinked polyacrylamide sieving used in conventional slab gel electrophoresis. Figure 1 illustrates the correlation between the two techniques. With CE-SDS, the separation can be done with less sample, fewer reagents and in a fraction of the time required for SDS-PAGE.

The CE-SDS Protein Kit contains two 50 µm ID capillaries and enough reagents to run 300 protein samples. Reagents include sample preparation buffer, dynamic sieving run buffer, protein size standards and an internal reference standard.

Automated

The BioFocus® capillary electrophoresis system combined with the CE-SDS Protein Kit provides fast, fully automated sample analysis and data handling. The dynamic sieving buffer in the capillary is replaced automatically prior to each analysis. No capillary conditioning time is required. Direct on-line UV detection provides peak migration times for molecular weight determination and peak areas for quantitation. This eliminates staining/destaining steps and the need for separate instrumentation for quantitation. The BioFocus Windows® software provides a graphical program for automatically computing molecular weights from the measured migration times.

Reproducible

Results obtained with the CE-SDS Protein Kit are not only quick and quantitative, but also highly reproducible. Table 1 shows the reproducibility statistics for the eight proteins contained in the CE-SDS Protein Size Standards.

Table 1. Reproducibility of CE-SDS

	Migration Time RSD%	Peak Area RSD%
Lysozyme	0.30	0.7
Trypsin inhibitor	0.35	1.0
Carbonic anhydrase	0.33	0.9
Ovalbumin	0.37	0.5
Serum albumin	0.39	1.0
Phosphorylase B	0.40	1.1
β-Galactosidase	0.43	1.0
Myosin	0.47	0.9

Normalized data, n=10

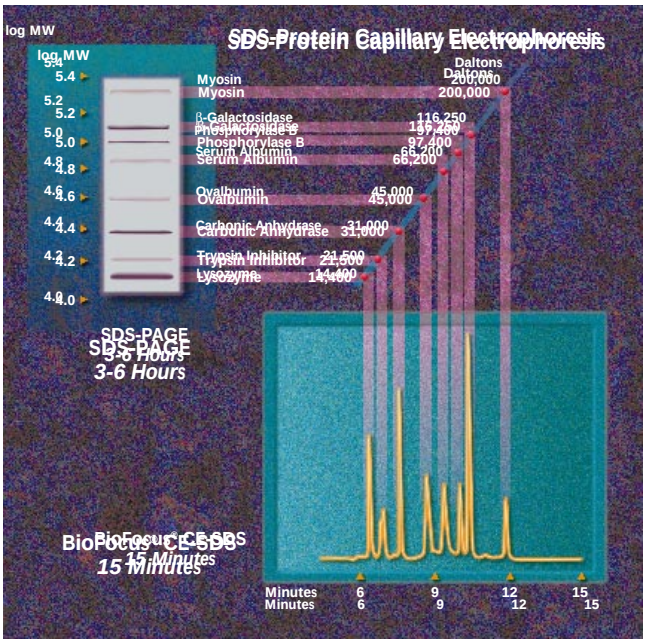

Fig. 1. The correlation between CE-SDS migration time and SDS-PAGE migration distance is illustrated above for the CE-SDS Protein Size Standards. Linearity of log molecular weight versus peak migration time allows generation of a calibration curve for estimating protein molecular weight.

Quantitative

Figure 2 shows the linearity of the integrated peak areas versus the concentration of a standard protein, carbonic anhydrase. The linear range of detector response is 0.5 to 1,000 µg/ml. Minimum detectable concentration is 0.36 µg/ml at a signal to noise ratio of 3.

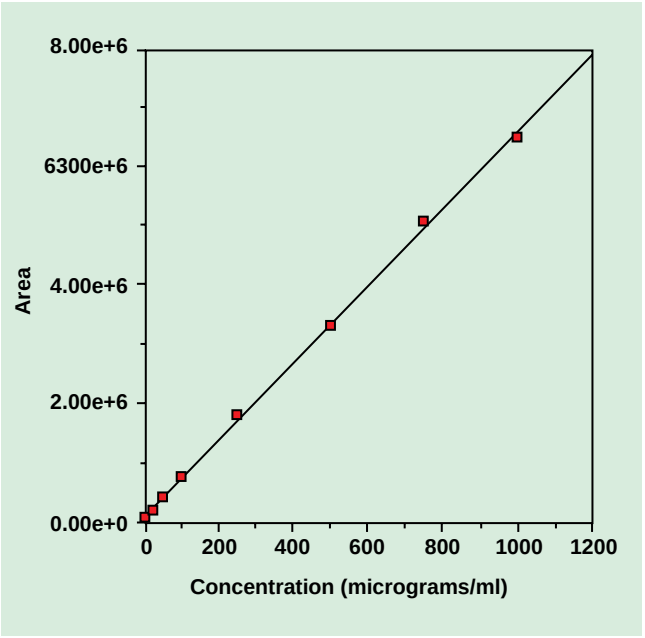

Fig. 2. The quantitative linear range of the CE-SDS Protein Kit extends from 0.5 to 1,000 µg/ml.

Applications

CE-SDS can be applied to a wide variety of protein separations and has been used for the analysis of enzymes, protein hormones, collagens, cereal proteins, immunoglobulins, protein conjugates, and recombinant protein pharmaceuticals. Figure 3 shows the separation of purified mouse monoclonal antibody (IgG_{2a}) under both reducing and non reducing conditions.

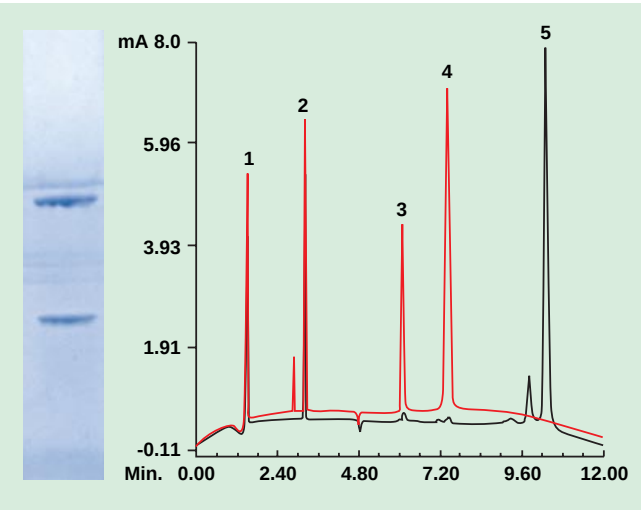

Fig. 3. The SDS-PAGE gel separation of IgG_{2a} under reducing conditions is analogous to the red line of the electropherogram overlay. The black line shows non reducing conditions. Peaks 1 and 2 in both runs are sodium azide preservative and benzoic acid internal standard, respectively. Peaks 3 and 4 are the light and heavy chain reduced form of the antibody, and peak 5 is the nonreduced form.

Ordering Information

Catalog Number	Product Description	Price
148-4160	**CE-SDS Protein Kit**	$375.00
148-5032	**CE-SDS Protein Run Buffer**	100.00
148-5033	**CE-SDS Protein Sample Buffer**	55.00
148-2015	**CE-SDS Protein Size Standard,** 14–200 kDa	90.00
148-2016	**CE-SDS Internal Reference**	25.00

Prices are good in the United States only, and are subject to change without notice. See the current Bio-Rad catalog for additional information.

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