Separation of SDS-Protein Complexes Using the CE-SDS Protein Kit

Catalog Numbers
148-4160
148-4161

BIO-RAD

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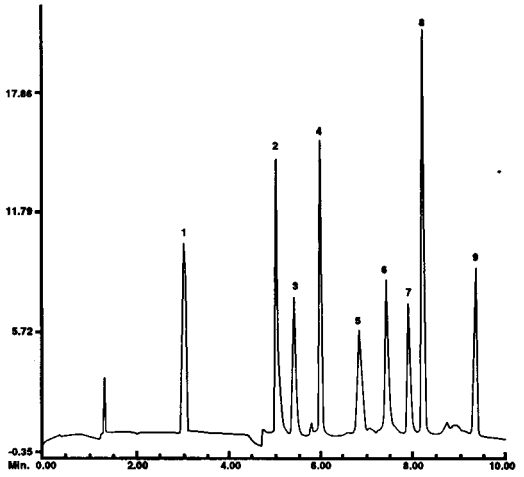
Section 1
Introduction

The CE-SDS Protein Kit separates SDS-protein complexes using a dynamic sieving mechanism. Incorporation of a hydrophilic polymer in the CE-SDS Protein Run Buffer causes a sieving effect so that SDS-protein complexes migrate according to their molecular size, with small proteins migrating faster and larger proteins migrating more slowly. The polymer type and concentration have been selected to provide a sieving range of approximately 14,000–200,000 M_r (Figure 1). This kit can be used to estimate protein molecular weights within this size range (Figure 2); molecular weight estimates outside of this range may not be accurate. Molecular weight estimates for glycoproteins obtained with this kit may also not be accurate. The kit can be used for quantitative determination of proteins in a sample based on peak area. The response curve shown in Figure 3 demonstrates that peak area is linear with protein concentration over a range from 0.5–1,000 µg/ml (note that final protein concentration in the prepared sample is plotted in this Figure).

IMPORTANT: Upon receipt of this kit, the CE-SDS Protein Size Standards should be stored at -20 °C and the remaining reagent solutions should be stored at room temperature.



Peaks

1. Benzoic acid (Reference Standard)
2. Lysozyme (14,400 Da)
3. Trypsin inhibitor (21,500 Da)
4. Carbonic anhydrase (31,000 Da)
5. Ovalbumin (45,000 Da)
6. Serum albumin (66,200 Da)
7. Phosphorylase B (97,000 Da)
8. β-Galactosidase (116,000 Da)
9. Myosin (200,000 Da)

Fig. 1. Separation of protein standards.

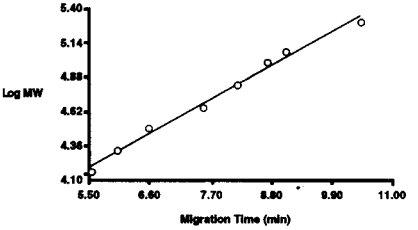


Fig. 2. Molecular weight calibration report.

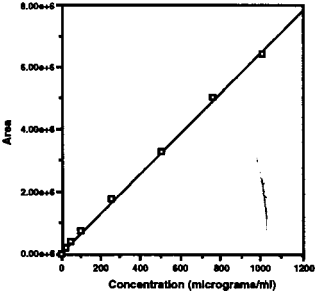


Fig. 3. Response linearity for carbonic anhydrase.

Section 2
CE-SDS Protein Kit Components

The CE-SDS Protein Kit contains all the supplies necessary to prepare and separate SDS-protein complexes using the BioFocus® capillary electrophoresis system. This kit comes in two parts:

148-4161 CE-SDS Buffer Module

- Two lengths of fused silica capillary, 40 cm x 50 µm ID x 375 µm OD, with detection window 10 cm from the end
- CE-SDS Protein Sample Buffer, 60 ml
- 0.1N NaOH, 30 ml
- 0.1N HCl, 30 ml
- CE-SDS Protein Run Buffer, 50 ml
- CE-SDS Internal Reference (benzoic acid, 1 mg/ml), 3 ml

148-2015 CE-SDS Protein Size Standard

- CE-SDS Protein Size Standards mixture containing 8 proteins (200 µl total volume, 20 mg/ml) shown in Figure 1

Section 3
Sample Composition

3.1 Protein Concentration

After preparation with the CE-SDS Protein Sample Buffer, total sample protein should not exceed 1 mg/ml. The mass binding ratio of SDS to protein is 1.4–1.6, and total final sample protein concentrations in excess of 1 mg/ml may not yield sufficient SDS binding.

3.2 Salt Concentration and Type

The maximum tolerable salt concentration in the sample depends upon the mode of injection. Pressure injection is less sensitive to salt, and salt concentrations of up to 200 mM have little effect on the separation. Electrophoretic injection is much more sensitive to salt, and salt concentrations above 50 mM reduce the loading efficiency in this injection mode.

The potassium salt of SDS has low solubility, and addition of the CE-SDS Protein Sample Buffer to samples containing potassium salts will result in precipitation of potassium-SDS. If the sample is known to contain potassium salts, it should be dialyzed against 25 mM Tris-HCl (pH 8.5) prior to addition of the CE-SDS Protein Sample Buffer.

Section 4
Sample Preparation

The sample preparation procedure quantitatively converts proteins in the sample to SDS-protein complexes. Dilute the sample 1:1 with the CE-SDS Protein Sample Buffer, then add CE-SDS Protein Internal Reference to a final concentration of 50 µg/ml. Place the sample in a 95–100 °C water bath for 10 minutes. After heating, cool the sample and centrifuge in a microcentrifuge for 2 minutes. Hold at room temperature prior to analysis. Samples and standard mixtures should be prepared fresh daily. If samples are to be analyzed in reduced form, a reducing agent should be added prior to heating; 2-mercaptoethanol (catalog number 161-0710) or dithiothreitol (catalog number 161-0613) can be used at final concentrations of 2.5% or 15 mM, respectively.

Section 5
Preparation of Protein Standards

The CE-SDS Protein Size Standard contains 100 mM dithiothreitol (DTT) and therefore the proteins are in reduced form; the final concentration of DTT in the prepared standard solution is 5 mM. Combine 10 µl of the CE-SDS Protein Size Standard solution, 100 µl CE-SDS Protein Sample Buffer, and 10 µl CE-SDS

Internal Reference. Add 80 µl deionized water to bring total sample volume to 200 µl. Hold the mixture in a capped 500 µl microcentrifuge vial in a 95–100 °C water bath for 10 minutes. Cool the mixture, centrifuge for 2 minutes in a microcentrifuge, then hold at room temperature prior to analysis.

IMPORTANT: Use of a contact heater block may not provide sufficient heat for quantitative SDS binding. Use of a water bath for sample and standard preparation is recommended.

Section 6
Capillary Type and Preparation

The capillaries supplied with this kit are 50 µm ID x 375 µm OD x 40 cm long. After removal from the protective TFE tubing, the capillary can be installed directly in the User Assembled Cartridge according to the instructions supplied with the BioFocus user assembled cartridge kit (catalog number 148-3052).

Because of the high viscosity of the sieving agent in the analysis buffer, use of a 50 µm ID capillary is required to obtain adequate purge volumes at 100 psi. Use of smaller ID capillaries would require much longer purge times, resulting in increased total analysis time, and would greatly increase the risk of capillary plugging. A capillary length of 24 cm provides good resolution and short analysis time.

Section 7
Analysis Conditions

Capillary	24 cm x 50 µm, (installed in user-assembled cartridge)
Polarity	Negative to positive
Buffer	CE-SDS Protein Run Buffer
Injection	10 kV for 5 seconds (electrophoretic) or 40 psi * seconds (pressure)
Run voltage	15 kV
Current limit	50 µA
Run time	15 min
Detection	220 nm at 0.01–0.05 AUFS
Cartridge temp.	20 °C
Carousel temp.	20 °C
Purge cycles (5)	90 sec with 0.1N NaOH 60 sec with 0.1N HCl 120 sec with CE-SDS Protein Run Buffer 0 sec with 1:1 dilution of CE-SDS Protein Sample Buffer 0 sec with 1:1 dilution of CE-SDS Protein Sample Buffer

See Figures 4 and 5 for a typical configuration and automation sequence for the BioFocus automated capillary electrophoresis systems.

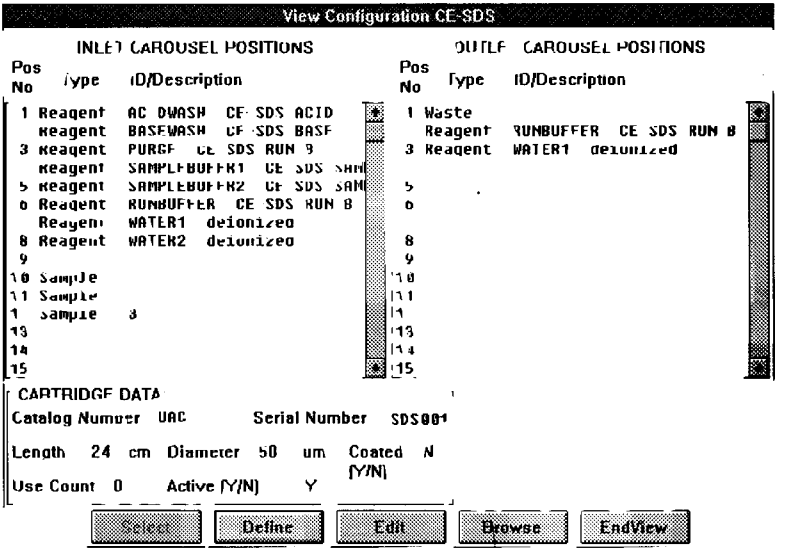


Fig. 4. Typical configuration for using the CE-SDS Protein Kit with the BioFocus automated capillary electrophoresis systems.

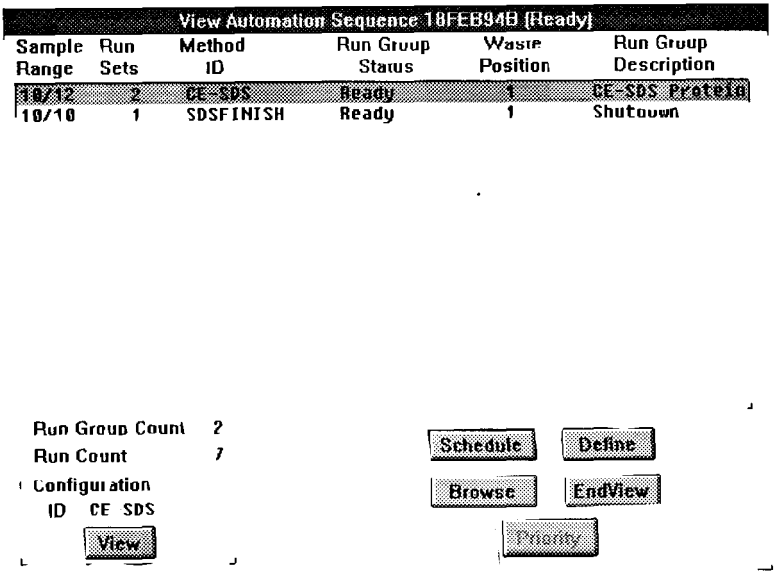


Fig. 5. Typical automation sequence for using the CE-SDS Protein Kit with the BioFocus automated capillary electrophoresis systems.

7.1 Buffer Preparation

The CE-SDS Protein Run Buffer should be degassed by centrifugation prior to use. After pipetting buffer into the purge and run buffer vials, centrifuge the vials for at least 2 minutes in a microcentrifuge immediately before inserting them into the BioFocus automatic sampler carousels.

7.2 Capillary Purge Sequence

For best results, the five-step purge sequence listed above is recommended. The first two purges with NaOH and HCl serve to sweep buffer and sample components from the previous run from the capillary. The third 120 second purge replenishes the capillary with fresh CE-SDS Protein Run Buffer. The final two 0 second purges with CE-SDS Protein Sample Buffer serve to wash any residual run buffer from the outer surface of the capillary prior to sample injection. Because the run buffer is very viscous, use of two different sample buffer vials is recommended for effective rinsing.

7.3 Detection Wavelength

Detection at 220 nm is recommended to achieve the optimal signal to noise ratio. At lower wavelengths (*e.g.* 200 nm) noise due to absorbance of buffer components reduces sensitivity, while at longer wavelengths (*e.g.* 280 nm) protein absorbance is reduced and noise is increased due to lower source light intensity.

7.4 Injection Mode

Best resolution and sensitivity are obtained using electrophoretic injection. However, in cases where the sample contains excessive amounts of salt, pressure injection may provide better results.

7.5 Operating Current

When operating the capillary at 15 kV constant voltage with cartridge temperature set at 20 °C, the current value is typically about 18 μA for a 24 cm capillary.

7.6 Analysis of Multiple Samples

For analysis of multiple samples, a fresh set of purge and run buffer vials should be used every ten injections. Also, installation of large-capacity vial holders (catalog number 148-6054) at the purge reagent and waste positions on the inlet and outlet carousels respectively is recommended. These vial holders accommodate 1.5 ml microcentrifuge vials (catalog number 223-9500) which have sufficient volume for ten samples.

IMPORTANT: When using sieving buffers containing polymers, the capillary tips should not be exposed to air for extended lengths of time. Exposure will cause drying, leading to capillary plugging. To minimize the risk of capillary plugging, the following precautions are recommended.

Program the BioFocus system to leave the buffer vials in the elevated position following analysis (check "Vials up at end of run" in Command menu), and program a Shutdown Method with NaOH and water purge cycles at the end of each automation sequence.

Place deionized water in the waste vial so the capillary outlet is immersed during purging (use 200 µl water for 500 µl waste vial or 500 µl water for 1.5 ml waste vial).

Section 8
System Shutdown

After use, the capillary should be washed with 0.1 N NaOH for 60 seconds and water for 120 seconds. This sequence may be automatically programmed at the end of an automation sequence using the shutdown method parameters shown below. This can be programmed using the Shutdown Method in the BioFocus software as a template.

Polarity	Negative to positive
Buffer	Deionized water
Injection	None
Run voltage	0 kV
Current limit	0.3 μA
Detection	220 nm
Run time	1.0 min
Cartridge temp.	20 °C
Carousel temp.	20 °C
Purge cycles (2)	60 seconds with 0.1N NaOH 60 seconds with deionized water
Options	Lamp turned off at end of run group

Section 9
Regenerating A Plugged Capillary

If the capillary becomes plugged (as evidenced by zero current, or failure of liquid to appear at the capillary outlet during manual purging) soaking the inlet and outlet ends in deionized water for 30 minutes to several hours will often dissolve the plug. If this fails, the following procedures may be successful; try procedure 1 first and, if unsuccessful, try procedure 2.

1. Immerse the capillary ends in hot (~70 °C, not boiling) deionized water for 10–15 minutes.
2. Immerse the capillary ends in a sonic bath filled with deionized water and sonicate for about 5–10 minutes.

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