SIGMA ELECTROPHORESIS™

SDS-PROTEIN CALIBRATION KIT
for Capillary Electrophoresis
Kit No. SDS-PRO-CE

January 1994 Technical Bulletin No. SDS-PCK1

INTRODUCTION

This kit is designed for separation of SDS-protein complexes using a non-gel sieving mechanism to determine molecular weights and protein purity on capillary electrophoresis units. The sieving range is 14,000-205,000.

Items Provided:
(Sufficient for approximately 300 runs.)

Item	Product No.	Amount
SDS-Protein Standards for Capillary Electrophoresis	M 2789	3.5 mg
SDS-Protein Separation Medium for Capillary Electrophoresis	M 6664	50 ml
SDS-2X Sample Buffer for Capillary Electrophoresis	S 9788	10 ml
SDS-Washing Solution	W 4253	100 ml
Orange G Solution-Internal Standard for Capillary Electrophoresis	O 9007	5 ml

Technical Bulletin No. SDS-PCK1

A. Protocol for Preparing SDS-Protein Standards and Unknown Protein Samples for Capillary Electrophoresis

1. SDS-Protein Standards

   a. Reconstitute the SDS-Protein Standards (Product No. M 2789) with 750 µl of SDS-2X Sample Buffer (Product No. S 9788) and 750 µl of deionized water. Vortex to assure the proteins are completely dissolved. Aliquot and freeze unused portions of solution at -20°C or below.

   b. To 100 µl of reconstituted SDS-Protein Standards from Step 1.a., add 3 µl of Orange G Solution-Internal Standard (Product No. O 9007) and 2.5 µl of 2-Mercaptoethanol (Product No. M 7154, not provided in kit).

   c. Boil the Protein Standards from Step 1.b. for 5 minutes. Wait until the solution has cooled before applying to a capillary.

2. Protein Sample

   a. Prepare protein sample(s) at a concentration of 0.2-1.0 mg/ml for each individual protein in the solution. The total protein concentration should not exceed 7 mg/ml.

   b. Mix the sample solution prepared in Step 2.a. in a ratio of 1:1 (v/v) with SDS-2X Sample Buffer (Product No. S 9788).

   c. To 100 µl of the sample solution prepared in Step 2.b., add 3 µl of Orange G Solution-Internal Standard (Product No. O 9007) and 2.5 µl of 2-Mercaptoethanol (Product No. M 7154, not provided in kit).

   d. Boil the sample solution prepared in Step 2.c. for 5 minutes. After it has cooled, apply to a capillary.

Notes:

1. **Salt concentrations must be below 50 mM for electrophoretic injection and below 200 mM for pressure injection.**
2. **Do not use any potassium salts since they will precipitate with SDS.**
3. **Omit the 2-Mercaptoethanol if the sample will be run under non-reducing conditions.**

B. Running Conditions

1 SDS-Protein Separation Medium (Product No. M 6664) and SDS-Washing Solution (Product No. W 4253) should be degassed before use.

2. Follow manufacturer's instructions to assemble the capillary to be used.

3. For an electrophoretic load use 10 kV for 20 seconds. For a pressure load use 5 psi for 12 seconds.

4. Run for 30 minutes at 15 kV constant voltage, negative to positive polarity, at a temperature of 20°C on a coated capillary (50 μm x 36 cm).

5. Detection System: Use 214 nm at 0.02-0.05 AUFS or follow manufacturer's instructions.

6. Purge Cycles:

   To prepare capillary prior to starting run:

Purge:	5	minutes with deionized water
	10	minutes with SDS-Protein Separation Medium (Product No. M 6664)

   Before each sample application:

Purge:	2	minutes with SDS-Washing Solution(Product No. W 4253)
	3	minutes with SDS-Protein Separation Medium (Product No. M 6664)
	0	seconds with deionized water (3X)

   After completion of run:

Purge:	2	minutes with deionized water
	3.5	minutes with SDS-Washing Solution (Product No. W 4253)
	2	minutes with deionized water
	3	minutes with dry nitrogen gas

C. Molecular Weight Determinations of Unknown Proteins using SDS-Protein Standards (Product No. M 2789)

1 Calculate the migration time for each protein in SDS-Protein Standards by subtracting the retention time of the internal standard (Orange G) from the retention time of each protein standard.

2. Plot the migration time for each protein standard vs. its molecular weight (See Table A) on semi-logarithm graph paper to obtain a linear standard curve.

3. Calculate the migration time for an unknown protein by subtracting the retention time for the internal standard (Orange G) from the retention time for the unknown protein.

4. Estimate the molecular weight of the unknown protein from the standard curve plotted in Step 2.

Table A. Subunit Molecular Weights of Proteins Contained in SDS-Protein Standards for Capillary Electrophoresis (Product No. M 2789)

PROTEIN	SUBUNIT MOLECULAR WEIGHT
α-Lactalbumin, Bovine Milk	14,200
Trypsin Inhibitor, Soybean	20,100
Carbonic Anhydrase, Bovine Erythrocyte	29,000
Ovalbumin, Chicken Egg	45,000
Albumin, Bovine Serum	66,000
Phosphorylase b, Rabbit Muscle	97,400
β-Galactosidase, *E. coli*	116,000
Myosin, Rabbit Muscle	205,000

Figure 1. Typical Electropherogram of SDS-Protein Calibration Standards using BioRad BioFocus 3000. 1) α-Lactalbumin, Bovine Milk; 2) Trypsin Inhibitor, Soybean; 3) Carbonic Anhydrase, Bovine Erythrocyte; 4) Ovalbumin, Chicken Egg; 5) Albumin, Bovine Serum; 6) Phosphorylase b, Rabbit Muscle; 7) β-Galactosidase, *E. coli*; 8) Myosin, Rabbit Muscle.