

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

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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.)

<u>Item</u>	Product No.	<u>Amount</u>
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

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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 μ l of SDS-2X Sample Buffer (Product No. S 9788) and 750 μ l 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 μ l of reconstituted SDS-Protein Standards from Step 1.a., add 3 μ l of Orange G Solution-Internal Standard (Product No. O 9007) and 2.5 μ l 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

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

- 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

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)

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
lpha-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, <i>E. coli</i>	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.

