APPENDIX E

Reagents for SDS-PAGE

1. Sample buffer (SDS reducing buffer)

The sample buffer was prepared as a stock solution by combination the following ingredients:

0.5 M Tris-HCL, pH 6.8	1.0 ml
Glycerol	2.0 ml
SDS (10% solution)	1.6 ml
0.05% Bromophenol blue	0.2 ml
2-6-mercaptoethanol	0.4 ml
UDW	2.8 ml

This mixture was stored at room temperature in small aliquots. One part of sample was diluted with equal part of the sample buffer and heated at 100°C for 4 min before loading into gel.

2. Tris-HCl (1.5 M, pH 8.8)

To prepare this solution, 18.15 g of Tris base (hydroxymethyl) amino-methane (USB Corporation, USA) was dissolved in 50 ml of UDW, then the pH was asjusted to 8.8 with 1 N HCl. The final volume was brought up to 100 ml with UDW. The solution was filtered through sterile a 0.2 μm membrane. This stock solution was stored at 4°C until use for preparing a working solution.

3. Tris-HCl (0.5 M, pH 6.8)

To prepare this solution, 6.05 g of Tris base (hydroxymethyl aminomethane) (USB Corporation, USA) was dissolved in 50 ml of UDW, then the pH was adjusted to 6.8 with 1 N HCl. The final volume was brought up to 100 ml with UDW. The solution was filtered through a sterile 0.22 μm membrane. This stock solution was stored at 4°C.

4. Sodium dodecyl sulfate (10% SDS; w/v)

This solution was prepared by dissolving 10 g of SDS (Bio-Rad, USA) in 100 ml of UDW.

5. Ammonium persulfate (10%; w/v)

This solution was prepared just before use by dissolving 50 mg of ammonium persulfate (Bio-Rad, USA) in 0.5 ml of UDW.

6. Separating gel (12%)

Polyacrylamide separating gel (12%) was prepared by mixing the following ingredients together:

1.5 M Tris-HCl, pH 8.8	2.5 ml
10% SDS solution	100 µl
30% Acrylamide/Bis, 29:1 ratio solution (Bio-Rad, USA)	4.0 ml
UDW	3.35 ml

The reagents were gently mixed and degassed under a vacuum for at least 5 min. The polymerization was initiated by adding 50 µl of the 10% ammonium persulfate (freshly prepared) and 5 µl of TEMED (Bio-Rad, USA). The gel was poured into the casting apparatus, over-layered with UDW and allowed to polymerize for at least 20 min at room temperature.

7. Stacking gel (4%)

The stacking gel (4%) was prepared by mixing the following reagents:

0.5 M Tris-HCl	2.5 ml
10% SDS solution	0.1 ml
30% Acrylamide/Bis, 29:1 ratio solution (Bio-Rad, USA)	1.3 ml
UDW	6.0 ml

All reagents were mixed gently and degassed under a vacuum for 15 min, then 50 µl of freshly prepared 10% ammonium persulfate and 10 µl of TEMED were subsequently added, respectively. After complete mixing and degassing, the upper portion of the gel polymerized in the casting apparatus was rinsed with UDW, the comb was inserted between the glass plates over the polymerized separating gel. The stacking gel was poured and allowed to polymerize for at least 45 min at room temperature before use.

9. Electrode (running) buffer (pH 8.3; 5 ×)

The buffer contained the following reagents: 15 g of Tris base (hydroxy-methyl) aminomethane (Sigma Chemical Co.); 72 g of glycine and 5 g of SDS. The buffer was prepared by dissolving all of the above reagents in a volume of UDW. After all ingredients were dissolved, the volume was made up to one liter with UDW. The buffer was stored at 4°C until use for preparing a working electrode (running) buffer.

10. Working electrode (running) buffer (1 ×)

Sixty ml of the 5× electrode buffer (Section 9) was diluted with 240 ml of UDW. Each preparation of the working running buffer was used for only one electrophoretic run.