APPENDIX C

Reagents for SDS-PAGE

1. Sample buffer (SDS reducing buffer)

The sample buffer was prepared as a stock solution by combining 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% Bromphenol blue	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 ^{o}C fro 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 adjusted to 8.8 with 1 N HCl. The final volume was brought up to 100 ml with UDW. The solution was filtered through a sterile 0.2 mm membrane. This stock solution was stored at 4^{o}Cuntil used 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 aminomethne) (USB Corporation, USA) was dissolved in 50 ml of UDW, then the pH was adjusted to 6.8 with 1N HCl. The final volume was brought up to 100 ml with UDW. The solution was filtered through a sterile 0.22 mm membrane. This stock solution was stored at 4^{o}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:

UDW	3.35 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml
10% SDS solution	100 µl
30% stock acrylamide solution	4 ml

The reagents were gently mixed and degassed under a vacuum for at least 5 min. The polymerization was initiated by adding 50 ml of the 10% ammonium persulfate (freshly prepared) and 5 ml 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 temprature.

7. Stacking gel (4%)

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

UDW	6.0 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml
10% SDS solution	100 µl
30% stock acrylamide solution	1.3 ml

All reagents were mixed gently and degassed under a vacuum for 15 min, then 50 µl of freashy prepared 10% ammonium persulfate and 10 µl of TEMED were subsequently added, respectively. After complete mixing, the upper portion of the gel polymerization in the casting apparatus was rinsed with UDW, the comb was inserted

between the glass plate over thepolymerized separating gel. The stacking gel was poured and allowed to polymerize for at least 45 min at room temperature befor use.

8. Stock electrode (running) buffer (5x)

The buffer contained the following reagents:

Tris base (hydroxy-methyl) aminomethane (Sigma Chemical Co., USA)	15 g
Glycine	72 g
SDS	5 g

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^{o}C until use for preparing a working electrode (running) buffer.

9. Working electrode (running buffer (1x))

Sixty ml of 5x electrode buffer was diluted with 240 ml of UDW. Each preparation of the working running buffer was used for only one electrophoretic run.

10. Staining of the gel

10.1 Coomassie Brilliant Blue staining (CBB)

Coomassie® Brilliant Blue R-250 dye (Sigma Chemical Co., USA) (2.5 g) was dissolved in 454 ml of absolute methanol befor 92 ml of glacial acetic acid and 454 ml of UDW were added. This dye was filtered through a Whatman No.1 paper and kept at room temperature.

10.2 Detstaining solution

10.2.1 High methanol destain solution

The solution was prepared by mixing 75 ml of glacial acetic acid, 457 ml od methanol and 25 ml of glycerol togethor. UDW was added to make 1,000 ml. The solution was kept at room temperature.

10.2.2 Standard (low-methanol) destain solution

The solution was prepared by mixing 75 ml of glacial acetic acid, 50 ml of methanol and 25 ml of glycerol together. UDW was added to make 1,000 ml. The solution was kept at room temperature.