APPENDIX A

Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis (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
10% SDS (w/v)	1.6	ml
0.5% Bromophenol blue (w/v)	0.2	ml
2-6-Mercaptoethanol	0.4	ml
Ultra-pure distilled water (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. Stock acrylamide solution (30%)

To prepare this solution, 30 g acrylamide (Bio-Rad, USA) and 0.8 g N, N'-methylene-bis-acrylamide (Bio-Rad, USA) were dissoved in 100 ml of UDW. The solution was sterilized by filtering through a sterile 0.22 μm membrane. This stock solution was stored at 4 °C in dark brown bottle.

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

To prepare this solution, 18.15 g Tris base (hydroxymethyl aminomethane) (Sigma Chemical Co., USA) was dissolved in 50 ml 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 0.22 μm membrane. This stock solution was stored at 4 °C until use for preparing a working solution.

4. 0.5 M Tris-HCl, pH 6.8

To prepare this solution, 6.05 g Tris base (hydroxymethyl aminomethane) (Sigma Chemical Co., 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 until use for preparing a working solution.

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

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

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

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

7. Separating gel (12%)

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

UDW	3.25 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml
10% SDS solution	100 μl
30% stock acrylamide solution	4.0 ml

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

8. Stacking gel (4%)

Polyacrylamide separating gel (4%) was prepared by mixing the following ingredients together:

UDW	6.0 ml
1.5 M Tris-HCl, pH 6.8	2.5 ml
10% SDS solution	100 μl
30% stock acrylamide solution	1.3 ml

The reagents were gently mixed and degassed under a vacuum for at least 5 min, then 50 µl 10% ammonium persulfate (freshly prepared) and 10 µl TEMED (Bio-Rad, USA) was subsequently added. 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 temparature before use.

9. 5 x Electrode buffer, pH 8.3

The buffer contained the following reagents:

Tris base (hydroxymethyl aminomethane)	15 g
Glycine	72 g
SDS	5 g

The buffer was prepared by dissolving all of the above reagents in a small volume of UDW. After all ingredients have been dissolved, the volume was made up to one liter with UDW. The buffer was stored at stored at 4 °C until use for preparing a working electrode (running) buffer.

10. 1 x Electrode (running) buffer

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

11. Staining of the gels

11.1 Coomassie Brillint Blue stain

Coomassie® Brilliant Blue R-250 dye (Sigma Chemical Co., USA) (2.5 g) was dissolved in 454 ml absolute methanol before 92 ml 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.

11.2 Destain solution

11.2.1 High methanol destaining solution

The solution was prepared by mixing 75 ml glacial acetic, 454 ml methanol and 25 ml of glycerol together. UDW was added to make 1,000 ml. The solution was kept at room temperature.

11.2.2 Standard (low-methanol) destaining solution

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

12. Silver stain (Commercial kit: Bio-Rad, USA)

Each Silver stain kit contains the following components:

12.1 Oxidizer concentrate

A ten fold stock solution contained potassium dicromate and nitric acid. It is stored at 4 °C until used.

12.2 Silver reagent concentrate

A ten fold stock solution contained silver nitrate. This solution was sensitive to heat; thus was stored at 4 °C until used.

12.3 Developer

Four bottles of dry chemical blend containing sodium carbonate and para-formaldehide were supplied in the test kit. They were stored at 4 °C until used.

Silver reagent preparation:

Oxidizer

Twenty ml oxidizer concentrate was mixed with 180 ml UDW. This solution was prepared on the day of staining.

Silver reagent

Twenty ml silver concentrate was added to 180 ml UDW. This preparation was also prepared on the day to be used.

Developer

It was prepared by dissolving 3.2 g developer provided in the kit in 100 ml of UDW. The content was dissolved for 15 min at 23 °C with continuous stirring. The solution was stored at 23-25 °C for not longer than one month.