APPENDIX D

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

1. Sample buffer (SDS reducing buffer)

The sample buffer was prepared as a stock solution by mixing 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	0.2	ml
2-6-Mercaptoethanol	0.4	ml and
Ultra-pure distilled water (UDW)	2.8	ml

This mixture was kept at 25°C in small aliquots. One part of sample was diluted with equal part of the sample buffer and heated at 100°C for 4 minutes before loading into gel.

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

To prepare this solution, Tris base (hydroxymethyl aminomethane) (USB, USA) (18.15 g) was dissolved in 50 ml of UDW, then the pH was adjusted to 8.8 with concentrate 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.

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

To prepare this solution, Tris base (6.05 g) was dissolved in 50 ml of UDW, then the pH was adjusted to 6.8 with concentrate 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.

4. Sodium dodecyl sulfate (10% SDS)

This solution was prepared by dissolving 10 g of SDS (USB) in the final volume of 100 ml of UDW to make 10% (w/v).

5. 10% ammonium persulfate (10% APS)

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.

UDW	3.4	ml
1.5 M Tris-HCl, pH 8.8	2.5	ml
10% SDS solution	100	µl and
30% Acrylamide/Bis solution, 29:1 (3.3% C)	4.0	ml

The reagents were gently mixed and degassed under vacuum for at least 5 minutes. The polymerization was initiated by adding 50 µl of 10% APS (freshly prepared) and 5 µl of TEMED (Bio-Rad). The gel was poured into the casting apparatus, overlaid with UDW and allowed to polymerize for at least 20 minutes at 25°C.

7. Stacking gel (4%)

Polyacrylamide stacking gel (4%) was prepared by mixing the following ingradients.

UDW	6.1	ml
0.5 M Tris-HCl, pH 6.8	2.5	ml
10% SDS solution	100	µl and
30% Acrylamide/Bis solution, 29:1 (3.3% C)	1.3	ml

The reagents were gently mixed and degassed under a vacuum for at least 5 min, then 50 µl of 10% APS (freshly prepared) and 10 µl of TEMED were subsequently added. The comb was inserted on the top of the glass plates. The stacking gel was poured and allowed to polymerize for at least 45 minutes at 25°C before use.

8. 5x electrode buffer, pH 8.3

The buffer contained the following reagents;

Tris base (hydroxymethyl amenomethane)	15	g
Glycine	72	g and
SDS	5	g

The buffer was prepared by dissolving all of the above reagents in a volume of UDW. After all ingredients have been dissolved, the volume was made up to one liter with UDW. The buffer was stored at 4°C.

9. 1x electrode buffer

Hundred milliliters of 5x electrode buffer was diluted with 400 ml of UDW.

10. Coomassie brilliant blue R-250 stain

Coomassie brilliant blue R-250 dye (Sigma Chemical Co.) (2.5 g) was dissolved in 454 ml of absolute methanol (Univar, USA) before 92 ml of glacial acetic acid (Merck) and 454 ml of UDW were added. The preparation was filtered through a Whatman No. 1 paper and kept at 25°C.

10.1 Destaining solution

10.1.1 High methanol destaining solution

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

10.1.2 Low methanol destaining 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 25°C.

11. Silver stain (Silver staining kit, Amersham BioScineces)

11.1 Fixing solution

This solution was prepared by mixing 100 ml of ethanol (Merck) with 25 ml of glacial acetic acid. The volume was made to 250 ml with UDW.

11.2 Sensitizing solution

The solution was prepared by mixing the following ingredients:

Ethanol	75	ml
Glutardialdehyde (25% w/v)	1.25	ml
Sodium thiosulfate	10	ml and
UDW to	250	ml

11.3 Developing solution

Sodium carbonate (6.25 g) was dissolved in 200 ml of UDW (vigorous stirring is required). Formaldehyde (37% w/v; 100 µl) was then added and the volume was made up to 250 ml with UDW.

11.4 Stop solution

This solution was prepared by dissolving 3.65 g of EDTA·$2H_2O$ in 250 ml of UDW.

11.5 2% glycerol solution

Glycerol solution was prepared by adding glycerol (20 ml) to 980 ml of DW.

12. Colloidal Coomassie brilliant blue stain (Neuhoff V., 1988) (for 1 page)

12.1 Fixing solution (freshly prepared)

Fixing solution was prepared by mixing o-phosphoric acid (85%) 0.5 ml, methanol 10 ml and 39.5 ml of DW.

12.2 Staining solution (freshly prepared)

12.2.1 Stock staining solution A

o-phosphoric acid (85%)	0.95	ml
Ammonium sulfate	4	g
and DW to	40	ml

12.2.2 Stock staining solution B

Coomassie brilliant blue G-250	0.05	g
DW to	1	ml

12.2.3 Working staining solution

Working solution was prepared by mixing 40 ml of solution A, 1 ml of solution B and 10 ml of methanol.

12.3 Neutralization solution

This solution was prepared by dissolving 6 g of Tris-base in 500 ml of DW, pH was adjusted to 6.5 with o-phosphoric acid.

12.4 Stabilizing solution

This solution was prepared by dissolving 100 g of ammonium sulfate in the final volume of 500 ml DW.