APPENDIX G

Reagents and solutions for SDS-PAGE

SDS-PAGE

1. Sample 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	1.6	ml
0.5% Bromophenol blue (w/v)	0.2	ml
Distilled water (DW)	2.8	ml
For reducing sample buffer,		
2-6-Mercaptoethanol	0.4	ml

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

2. Stock acrylamide solution (30%)

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

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

To prepare this solution, 18.15 g Tris-base (hydroxymethyl aminomethane) was dissolved in 50 ml DW, then the pH was adjusted to 8.8 with 1 N HCl. The final volume was brough up to 100 ml with DW. The solution was filtered through a sterile 0.22 μm filter membrane and stored at 4°C until use.

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

To prepare this solution, 6.05 g Tris-base (hydroxymethyl aminomethane) was dissolved in 50 ml DW, then the pH was adjusted to 6.8 with 1 N HCl. The final volume was brough up to 100 ml with DW. The solution was filtered through a sterile 0.22 μm filter membrane and stored at 4°C until use.

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

This solution was prepared by dissolving 10 g of SDS powder in 100 ml of DW. The solution was filtered through filter membrane (Whatman no. 2).

6. Ammonium persulfate (10% w/v)

This solution was prepared just before use by dissolving 50 mg of ammonium per sulfate in 0.5 ml DW.

7. Resolving gel (12%)

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

DW	3.25	ml
Tris-HCl, pH 8.8 (1.5 M)	2.5	ml
SDS solution (10%)	0.1	ml
stock acrylamide solution (30%)	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 of 10% ammonium persulfate (fresh prepared) and 5 µl of TEMED. The gel was poured into the gel casting apparatus, over-layered with DW and allowed to polymerize for at least 30 minutes at 25°C.

8. Colloidal Coomassie Brilliant Blue stain

This solution was prepared as described in section 5.10 of **Appendix F.**