APPENDIX 4

REAGENTS USED FOR SDS-PAGE

Solution A
Acrylamide 22.2 g
N, N'- Methylene bis-acrylamide 0.6 g
Volume made up to 100 mL with distilled water and stored at 4°C in amber colour bottle.

Solution B
Tris 18.15 g
SDS 0.48 g
Volume made up to 100 mL with distilled water and pH adjusted to 8.8 using HCl

Solution C
Ammonium per sulfate solution
Ammonium per sulfate 0.1 g dissolved in 1 mL distilled water (freshly prepared before use).

Solution D
Tris 6.05 g
SDS 0.48 g
Volume made up to 100 mL with distilled water and pH adjusted to 6.8 using HCl

Electrophoresis buffer
Tris 6.0 g
Glycine 28.8 g
SDS 1.0 g
Volume made up to 1000 mL with distilled water and pH adjusted to 8.3.
Sample buffer

- Glycerol: 2.0 mL
- 10% SDS: 5.0 mL
- Mercapto ethanol: 1.0 mL
- 0.1% Bromo phenol blue: 0.4 mL
- Solution B: 1.6 mL

Silver staining

Fixing

Acetic acid

Sensitization

Glutaraldehyde (10%)

Silver impregnation

Silver nitrate (0.1%)
Dithiothreitol (5 ng/mL)

Development

3% Sodium carbonate
0.019% Formaldehyde

Stopping and preservation

- Sodium citrate: 2.3 M
- Glycerol: 1.5%