APPENDIX

1) Buffers, reagents and solutions for agarose gel electrophoresis

(i) Agarose gel loading dye (6x)

Glycerol 1750µl
10% Bromophenolblue 25µl
10% XyleneCyanol 25µl
Distilled water 5000µl

(ii) Ethidium Bromide (10mg/ml)

Ethidium Bromide 0.2 gms
Distilled water 20 ml
Mix well and store at 4°C in dark.

(iii) Tris Borate EDTA buffer (TBE buffer) (10X stock)

Tris Base 216.0 gms
Boric acid 110.0 gms
EDTA 11.6 gms
Distilled water 2000 ml
pH 8.3

The TBE buffer was diluted 10 times before use.

2) Luria Bertani Broth (LB medium)

Yeast extract 5 gms
Tryptone 10.0 gms
Sodium Chloride 10.0 gms
Distilled water 1000 ml
pH 6.9-7.1

Media was sterilized by autoclaving.
3. Buffers for affinity purification of mAbs with MAbSelect SuRe resin

(i) Buffer A (pH 7.2)

Sodium phosphate 2.8 gm
Sodium chloride 8.7 gm
Distilled water to 1000 ml
pH 7.2

(ii) Buffer B (pH 3.0)

Sodium citrate 29.4 gm
Distilled water to 1000 ml
pH 3.0

(iii) 1M Tris (pH 8.0)

Tris base 60.57 gm
Distilled water to 50 ml
pH 8.0

These buffers were filtered through 0.2µm filter and stored at 4°C until use.

4) Buffers, reagents and solutions for SDS-PAGE and Western blot

(i) Resolving gel (12%, for 5 ml)

Acrylamide-Bisacrylamide mix (30%) 2.0 ml
1.5M Tris (pH 8.8) 1.3 ml
SDS (10%) 0.05 ml
APS (10%) 0.05 ml
TEMED                    0.002 ml  
Distilled water          1.6 ml  

(ii) Stacking gel (5%, for 2 ml) 

Acrylamide-Bisacrylamide mix (30%)  0.33 ml  
1.0M Tris (pH 6.8)           0.25 ml  
SDS (10%)                   0.02 ml  
APS (10%)                   0.02 ml  
TEMED                      0.002 ml  
Distilled water            1.4 ml  

(iii) Running buffer (Tris-Glycine buffer, pH 8.3, 5X) 

Tris base                  15.1 gm  
Glycine                    94 gm   
SDS (10%)                  50 ml   
Distilled water to         1000 ml 

pH 8.3                     

The buffer was stored at room temperature and diluted 5 times before use. 

(iv) Reducing Sample buffer (3X) 

SDS                       3 gm   
2-mercaptoethanol (14M)    3 ml   
Bromophenol blue           0.01 gm 
Sucrose                    20 gm  
1M Tris (pH 6.8)           6.25 ml
Distilled water to 50 ml

The buffer was frozen in small aliquots at -20°C and diluted 3 times before use.

(v) Coomassie Staining Solution

Methanol 50%
Acetic acid 10%
Distilled water 40%

0.1g of brilliant blue is added to 50 ml of staining solution

(vi) SDS PAGE destaining solution

Methanol 50%
Acetic acid 10%
Distilled water 40%

Silver staining

(vii) Fixer-silver staining

Methanol 250 ml
Acetic acid 25 ml
Distilled water to 500 ml

The solution was stored at room temperature until use.

(viii) Sensitizer-silver staining

Sodium thiosulphate 0.1 gm
Distilled water to 500 ml

The solution was stored at room temperature until use.

(ix) Silver nitrate staining solution

Silver nitrate 0.5 gm
Distilled water to 500 ml
The solution was stored at room temperature until use.

(x) **Developer-silver staining**

- Formalin \(541 \mu l\)
- Sodium carbonate \(10 \text{ gm}\)
- Distilled water to \(500 \text{ ml}\)

The solution was stored at room temperature until use.

(xi) **Stopper-silver staining**

- Acetic acid \(5 \text{ ml}\)
- Distilled water to \(500 \text{ ml}\)

The solution was stored at room temperature until use.

**Western blot buffer**

(xii) **Protein transfer buffer (1x)**

- Tris base \(3.03 \text{ gms}\)
- Glycine \(14.4 \text{ gms}\)
- Methanol \(200 \text{ ml}\)

Make up the volume to \(1000 \text{ ml}\) with distilled water.

(xiii) **Blocking solution for western blot**

3% Skimmed milk powder in 100 ml Phosphate buffered saline with 0.05% Tween (PBST) solution.

5. **Buffers, reagents and solutions for ELISA**

(i) **Carbonate – bicarbonate buffer (pH 9.6)**

- Sodium carbonate \((\text{Na}_2\text{CO}_3)\) \(1.59 \text{ g}\)
- Sodium bicarbonate \((\text{NaHCO}_3)\) \(2.93 \text{ g}\)
- Distilled water \(1000 \text{ ml}\)
pH 9.6

The coating buffer was stored at 4°C.

(ii) Phosphate buffered saline (PBS, pH 7.2)

Sodium chloride (NaCl) 8.00 g
Potassium chloride (KCl) 0.20 g
Potassium dihydrogen ortho
phosphate (KH$_2$PO$_4$) 0.20 g
Disodium hydrogen phosphate
(Na$_2$HPO$_4$, 12 H$_2$O) 1.16 g
Distilled water 1000 ml
pH 7.2

(iii) ELISA washing buffer (PBST)

Phosphate buffered saline (PBS) 1000 ml
Tween-20 (0.05% v/v) 500 µl

(iv) ELISA blocking solution

Bovine gelatin 1 g
PBS Tween-20 100 ml

The blocking solution was warmed at 37°C for complete
dissolution of gelatin and freshly prepared before use.

(v) Citrate buffer (pH 5.0)

Citric acid monohydrate 5.11 g
Disodium hydrogen phosphate 7.3 g
Distilled water 1000 ml
pH 5.0

(vi) Substrate solution

Tetra methyl benzidine tablet (10 mg) 1 tablet
Sodium citrate buffer (pH 4.2) 10 ml
Hydrogen peroxide (35%) 3 µl

The substrate solution was prepared fresh as and when required and used immediately after preparation.

(vii) Stopping solution

Sulphuric acid (1.25 M) 68 ml
Distilled water 932 ml

6. Buffers for mAb coupling and immuno-affinity chromatography

(i) Coupling buffer

Sodium bicarbonate 8.4 gm
Sodium chloride 4.61 gm
Distilled water to 500 ml
pH 8.3

The coupling buffer was prepared freshly before use.

(ii) Blocking buffer

Tris-HCl 6.06 gm
Distilled water to 500 ml
pH 8.5

The blocking buffer was prepared freshly before use
(iii) **Coupling wash buffer**

- Sodium acetate: 4.1 gm
- Sodium chloride: 14.61 gm
- Distilled water to: 500 ml
- pH 4.5

The coupling wash buffer was prepared freshly before use.

(iv) **Elution buffer**

- Glycine: 3.75 gm
- Distilled water to: 500 ml
- pH 2.5

(v) **Neutralizing buffer**

- Tris-HCl: 60.5 gm
- Distilled water to: 500 ml
- pH 8.6