

## **APPENDIX**

**XI. APPENDIX****PBS (phosphate buffered saline) 1000 ml:**

NaCl	80 g
KCl	2 g
Na <sub>2</sub> HPO <sub>4</sub>	11.5 g
KH <sub>2</sub> PO <sub>4</sub>	2 gm
Distilled water	1000 ml
pH adjusted to 7.2.	

**Citrate Buffer (10mM) 1000 ml:**

Citric acid	2.1 gm
Distilled water	Up to 1000 ml
pH adjusted to 6.0	

**Protein Lysis solution (500µl):**

1M Tris (pH 7.4)	10 ul
5M NaCl	15 ul
0.5 M EDTA (pH 8.0)	5 ul
Triton-X 100	5 ul
Glycerol	50 ul
Distilled water	415 ul

**Protease inhibitor cock tail (for 1 ml lysis solution):**

1M Dithiothreitol (DTT)	1ul
1mg/ml Aprotinin	4 ul
5mg/ml Leupeptin	1ul
200mM Phenyl Methyl Sulfonyl fluoride (PMSF)	5ul

**Acrylamide and N, N'-methylene bisacrylamide (36:1):**

Acrylamide	25 gm
Bisacrylamide	0.68 gm
Distilled water	85.57 ml
Filtered and stored at 4°C	

**SDS Gel loading Buffer (5X Sample buffer) (10 ml):**

Tris- Cl (pH-6.8)	1.75 ml
Glycerol	3 ml
SDS	1.028 g
DTT	0.93 g
Bromophenol blue	1.2 mg
Distilled Water	Up to 10 ml

**Stacking gel components (4%):**

Water	3.4 ml
Acrylamide (30%)	0.82 ml
0.5 M Tris (pH -6.8)	0.62 ml
SDS (10%)	0.05 ml
APS (10%)	0.05 ml
TEMED	0.005 ml

**Resolving gel components (10%):**

Water	4.0 ml
Bis acrylamide (30%)	3.3 ml
1.5M Tris (pH 8.8)	2.5 ml
SDS (10%)	0.1 ml
APS (10%)	0.1 ml
TEMED	0.004 ml

**Tris –glycine electrophoresis buffer (5 X, 1000ml):**

Tris base	15 gm
Glycine	72 gm
SDS	5 gm
Distilled water	up to 1000 ml

**Transfer buffer (1000 ml):**

Tris	3 g
Glycine	14.4 g
SDS (10%)	0.3 ml
Methanol	0.2 ml
Distilled water	Up to 1000 ml

**Tris buffered saline Tween-20 (TBST) 100 ml:**

1M Tris (pH 7.4)	2 ml
5M NaCl	3 ml
Tween-20	1 ml
Distilled water	Up to 100 ml

**TNM Buffer (1000ml):**

Tris base	3.63 gm
NaCl	1.75 gm
MgCl <sub>2</sub>	3.04 gm
Distilled Water	Up to 1000 ml
pH adjusted to 9.5	

**TE (Tris EDTA) buffer (100 ml) :**

10mM Tris	0.12g
1 mM EDTA	0.037g
Distilled Water	100 ml
pH adjusted to 7.5	

**Tris-Borate EDTA Buffer (10 X) 1000 ml:**

Tris	108 g
Boric acid	55 g
0.5 M EDTA	40 ml
Distilled water	Up to 1000 ml
pH adjusted to 8.0	

**Lysis solution for DNA isolation for PCR (1 ml)**

1 M Tris	0.01 ml (0.01M Tris HCl)
0.5 M EDTA	0.01 ml (0.005M EDTA)
5 M NaCl	0.04 ml (0.2M NaCl)
Distilled Water	0.91 ml
10 % SDS	0.01 ml (1%)
Proteinase K (10mg/ml)	0.02 ml (200 µg/ml)

**Lysis solution for DNA isolation for Laddering assay (1 ml)**

100 mM Tris	0.01 ml (50 mM)
100 mM EDTA	0.01 ml (20 mM)
Triton-X-100	0.01 ml (1%)
Distilled Water	0.94 ml
10 % SDS	0.01 ml (1%)
Proteinase K (10mg/ml)	0.02 ml (200 µg/ml)