VIII. APPENDIX

Useful recipes

Culture Media

1. Brain heart infusion (BHI) agar

BHI agar (dehydrated)	52.00 g
Distilled water	1,000.00 ml

Melt, disperse in tubes and autoclave at 121 °C 15 lbs for 15 min. Allow tubes cool in slant position. Or, after autoclave, pour plates and allow cool.

2. BHI broth

BHI	37.00 g
Distilled water	1,000.00 ml

Dissolve, disperse 30 ml in each 125 ml-Erlenmeyer flask. Autoclave at 121 °C 15 lbs for 15 min.

3. Luria-Bertani (LB) Medium

LB	20.00 g
Deionized H_2O up to	1,000.00 ml

Shake until the solutes have dissolved. Sterilize by autoclaving for 20 min at 15 lbs on liquid cycle.

4. Sabouraud Dextrose Agar (SDA)

Dehydrated SDA agar (Becton Dickinson)	65.00 g
Distilled water	1,000.00 ml

Suspend 65 g of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Autoclave at 121 °C 15 lbs. for 15 min.

Reagents and buffers

1. **Phosphate buffer saline (9 mM sodium phosphate, 27 mM sodium chloride, pH 7.2)**

Na_2HPO_4	575.00 mg
NaH_2PO_4 (anhydrous)	100.00 mg
NaCl	800.00 mg

Dissolve reagents in 400 ml distilled water and adjust pH to 7.2 with 1N HCl or 1N NaOH, and then fill distilled water up to 500 ml. Store at 4 °C.

2. **PBT-Tween (PBS-T)**

Phosphate buffer saline pH 7.2	100.00 ml
Tween 20	0.05 ml

3. **Amido black 10B, 0.1%**

Amido black 10B	0.10 g
Acid-ethanol solution	100.00 ml
Acid-ethanol solution containing;	
Absolute Ethanol	25.00 ml
Acetic acid	10.00 ml
Distilled water up to	100.00 ml

Stain nitrocellulose membrane for 1 min and destain with acid ethanol solution for 30-60 min.

4. **Blocking buffer**

Non-fat dry milk	5.00 g
PBS, pH 7.2	100.00 ml

Prepare fresh daily.

5. Coomassie brilliant blue R-250, 0.1%

Coomassie brilliant blue R-250	0.10	g
Acid-methanol solution	100.00	ml
Acid-methanol containing;		
Methanol	40.00	ml
Acetic acid	10.00	ml
Distilled water up to	100.00	ml

Stain polyacrylamide gel for 30-60 min and destain with acid-methanol solution for 1-3 h by several change volume.

6. Chloronapthol solution (Chromogen stock solution)

4-Chloro-1-napthol	0.30	g
Absolute ethanol	10.00	ml

Store at –20 °C

7. Working chromogenic substrate solution containing

Stock chloronapthol solution	0.10	ml
50 mM Tris-HCl, pH 7.6	10.00	ml

Remove the white precipitation by filtering through Whatman no.1 filter paper. Before using add 10 µl of 30% H_2O_2.

8. 500 mM Ethylene diamine tetra acetic acid (EDTA) pH 8.5

EDTA (sodium salt, dehydrate)	18.60	g
Distilled water	100.00	ml

Effective concentration to inhibit mettalo-proteases is 1 mM. Stable for months at 4 °C.

9. 50mM Iodoacetic acid (IAA)

Iodoacetic acid (sodium salt)	1.04 g
Distilled water	100.00 ml

Effective concentration to inhibit serine-proteases is 10-50 μM. Stable for months at -20 °C. Decomposes slowly should be prepared freshly.

10. 50 mM Phenylmethanesulphonyl fluoride (PMSF)

Phenylmethanesulfonyl fluoride	0.87 g
Methanol	100.00 ml

Effective concentration to inhibit cysteine-proteases is 0.1-1 mM. Stable at least 9 months at 4 °C.

11. 0.1% Merthiolate

Merthiolate (Thiomerosol; Sigma)	1.00 g
Sodium tetraborate	0.74 g
Distilled water	100.00 ml

Store at 4 °C in screwcapped dark brown bottle.

12. 30%T 2.6%C Acrylamide solution

Acrylamide	29.20 g
N',N'-bis-methylelne-acrylamide	0.80 g
Distilled water up to	100.00 ml

Store in screwcapped dark brown bottle. Stable at least 1 month at 4 °C.

13. 10% Ammonium persulfate (APS)

Ammonium persulfate	0.05 g
Distilled water	100.00 ml

Prepare fresh daily.

14. 0.1% Bromphenol blue tracking dye

Bromphenol blue	0.10 g
Ethanol	100.00 ml

Store at RT.

15. Electrode reservoir buffer solution (5X Running buffer), pH 8.3

Tris base	15.00 g
Glycine	72.00 g
SDS	5.00 g
Distilled water up to	1,000.00 ml

Do not adjust pH with acid or base. Store at 4 °C.

16. Sample buffer (2X reducing buffer)

SDS	1.00 g
Glycerol	2.00 g
0.1% Bromphenol blue	2.00 g
1M Tris-HCl, pH 6.8	1.25 ml
2-β-mercaptoethanol	1.00 ml
Distilled water up to	10.00 ml

Store at 4 °C and should be prepared fresh weekly.

17. 10% Sodium dodecyl sulphate (SDS)

SDS	1.00 g
Distilled water	10.00 ml

Store at room temperature and should be prepared fresh weekly.

18. Separating gel buffer (1.5 M Tris-HCl, pH 8.8)

Tris base	18.15 g
Distilled water	60.00 ml

Dissolve and adjust to pH 8.8 with 5 N HCl, making up to 100 ml with distilled water. Store at 4 °C.

19. Stacking gel buffer (0.5 M Tris-HCl, pH 6.8)

Tris base	6.05 g
Distilled water	60.00 ml

Dissolve and adjust to pH 6.8 with 5 N HCl, making up to 100 ml with distilled water. Store at 4°C.

20. Transfer buffer, pH 8.3

Tris base	3.03 g
Glycine	14.40 g
Methanol	200.00 ml
Distilled deionized water up to	1,000.00 ml

Do not adjust pH with acid or base. Store at 4°C.

24. 50X Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris base	242.00 g
Glacial acetic acid	57.10 ml
0.5 M EDTA pH 8.0	100.00 ml

Dissolve reagents in 500 ml distilled water and then fill distilled water up to 1,000 ml. Store at RT

25. Tris-EDTA buffer (TE pH 8.0)

1 mM Tris-Cl (pH 8.0)

0.1 mM EDTA (pH 8.0)

Sterilize the solution by passage through a 0.22-micron filter. Store at 4 °C.

Alkaline lysis buffers for minipreparations of plasmid DNA

1. Resuspension buffer (Solution I)

25 mM Tris-Cl (pH 8.0)

10 mM EDTA (pH 8.0)

Solution I can be prepared in batches of approximately 100 ml, autoclaved for 15 min at 10 lb/sq.in. on liquid cycle, and stored at 4 °C.

2. Lysis buffer (Solution II)

0.2 N NaOH (Freshly diluted from a 10 N stock)

1% SDS

NaOH pellets (8.0 g) were dissolved in distilled water and 50.0 ml of 20% SDS solution was added. The final volume was adjusted to 10 litter.

3. Neutralization buffer (Solution III)

5 M potassium acetate	60.00 ml
Glacial acetic acid	11.50 ml
H_2O	28.50 ml

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

Reagents for purification GST-fusion protein

1. **Binding buffer (1X PBS pH 7.3: 140 mM Nacl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4)**

Nacl	7.62 g
KCl	0.20 g
Na_2HPO_4	1.42 g
KH_2PO_4	0.25 g

Dissolve reagents in 800 ml distilled water and adjust pH to 7.3 with 1N HCl or 1N NaOH, and then fill distilled water up to 1,000 ml. Store at 4 °C.

2. **Elution buffer pH 8.0 (50 mM Tris-HCl and 10 mM reduced glutathione)**

Tris-HCl	7.88 g
Reduced glutathione	3.07 g

Dissolve reagents in 800 ml distilled water and adjust pH to 8.0 with 1N HCl or 1N NaOH, then fill distilled water up to 1,000 ml, and Sterilize the solution by passage through a 0.22-micron filter. Store at 4 °C.

IX. CURRICULUM VITAE

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