APPENDIX A

1. Protein concentration determination

The concentration of protein was determined by Lowly's method. Protein standard BSA was diluted in distilled water to concentrations of 0.2, 0.5, 0.8, 1.2, 1.5 mg/ml. A volume of 100 ul of either protein standard or protein samples was mixed with 4.5 ml of Lowly's reagent (Bio-Rad). The absorbance at 750 nm was determined from protein standard. The concentration of protein sample was calculated by referring to the concentration of protein standard.

2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The method of SDS-PAGE was performed as described by Laemmli, 1970. The gel solution was prepared as shown in Table 4. Electrophoresis was carried out in the descending direction on the Tris-glycine buffer (25 mM Tris-HCl, pH 6.8, 192 mM glycine and 0.1% (w/v) SDS) using a constant 100 V for 30 min and 200 V for 2 h or until the tracking dye reached the edge of the gel.

Table 4. Preparation of SDS-Polyacrylamide gel

Solution	4% Stacking gel	12% Resolution gel
Water	1.88 ml	3.23 ml
40% Acrylamide(acrylamide: *N,N'*-methylenebisacrylamide, 29:1)	0.3 ml	2.1 ml
1.5 M Tris-HCl, pH 8.8	-	1.52 ml
0.5 M Tris-HCl, pH 6.8	0.75 ml	-
10% SDS	0.03 ml	0.07 ml
10% APS	0.03 ml	0.07 ml
TEMED	0.01 ml	0.01 ml

SDS = Sodium Dodecyl Sulfate

APS = Ammonium Persulfate

TEMED = N,N,N,'N'-tetramethylenediamine

APPENDIX B

1. Chemical stock solution and buffer

5 M Tris-HCl

Tris-base	6	g
Distilled water	100	ml

Adjust the pH to 8.8 by adding concentrated HCl. Adjust the volume of the solution to 100 ml with distilled water and sterilize by autoclaving.

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Tris-base	6	g
Distilled water	100	ml

Adjust the pH to 6.8 by adding concentrated HCl. Adjust the volume of the solution to 100 ml with distilled water and sterilize by autoclaving.

10% SDS (w/v):

SDS	10	g
Distilled water	90	ml

Heat to 68 $^{\circ}$C and stir with a magnetic stirrer to assist dissolution. Adjust the volume to 100 ml with distilled water and store at room temperature. Sterilization is not necessary.

0.1% APS (w/v):

Ammonium persulfate	1	g
Distilled water	10	ml

Phosphate-buffered Saline (PBS):1X

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.44	g
KH2PO4	0.24	g

Dissolve the ingredients in 800 ml of distilled water. Adjust the pH to 7.4 with HCl. Add distilled water to 1000 ml. Sterilize the buffer by autoclaving and store at room temperature or 4 $^{\circ}$C

Ponceau S

Ponceau S	10	g
Acetic acid	50	ml
Deionized water to	1000	ml

0.5% Trypsin

Trypsin	0.5	g
EDTA	0.02	g
1xPBS	100	ml

SDS lysis buffer

2% (w/v) SDS

50 mM Tris, pH6.8

10% glycerol

2. Solutions for electrophoresis

Tris-glycine buffer, Running buffer: 10X

Tris base	30.28	g
Glycine	144.13	g
SDS	10	g

Dissolve the ingredients in 800 ml of distilled water. Adjust he volume to 1000 ml with distilled water.

SDS Gel loading buffer: 4X

200 mM Tris-HCl, pH 6.8

8% (w/v) SDS

0.4% (w/v) bromophenol blue

40% (v/v) glycerol

8% (v/v) 2-B-mercaptoethanol

400 mM DTT

Adjust the volume of the solution to 50 ml with distilled water and store at –80°C

Comassie blue staining

Comassie Brillant Blue R250	0.025	g
Methanol	43	ml
Acetic acid	7	ml
Distilled water to	100	ml

Destain solution

Methanol	30	ml
Acetic acid	7	ml
Distilled water	63	ml

3. Solution for Western blot analysis

Electroblotting buffer: 1X

Tris-base	5.8	g
Glycine	7.9	g
Methanol	200	ml

Dissolve the ingredients in distilled water and bring up to volume 1000 ml with distilled water.

TBS-T buffer: 5X

Tween$^{@}$20	5	ml
NaCl	45	g
Tris-HCl	31.75	g
Tris-base	5.8	g

Dissolve the ingredients in distilled water and bring up to volume 1000 ml with distilled water.

Blocking buffer:

Low fat dry milk	5	g
1X TBS-T	100	ml

Washing buffer:

Low fat dry milk	10	g
1XTBS-T	1000	ml

Stripping buffer

100 mM β-mercaptoethanol (14.3 M stock)

2% (w/v) SDS 10 ml

62.5 mM Tris-HCl, pH 6.8

Incubate the membrane at 60°C 30 min and then wash with TBS-T, 2x10 min.