APPENDIX A

Reagents and buffers for agarose gel electrophoresis

1. Loading dye (10x)

Ten times concentrate loading dye (10x) consisted of 50% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanole FF.

2. TAE buffer (Tris-acetate/EDTA electrophoresis buffer; 50x)

To prepare 50x TAE buffer, following ingredients were mixed:

Tris-base	242	g
EDTA•$2H_2O$	18.16	g
Glacial acetic acid	57.1	ml
UDW	700	ml

The pH of this buffer was adjusted to 8.3 with glacial acetic acid the volume was made up to 1,000 ml and sterilized by autoclaving.

3. Working (1x TAE)

To prepare the 1x TAE buffer, 20 ml of 50x TAE was added to 980 ml of UDW. This solution can be reused three times.

4. TBE buffer (5x)

To prepare the 5x TBE buffer, the following ingredients were mixed:

Tris-base	52	g
Boric acid	27.5	g
EDTA•$2H_2O$	4.65	ml
UDW	700	ml

The solution was adjusted pH to 8.3 with concentrate HCl before the volume was made to 1,000 ml. This buffer was sterilized by autoclaving.

5. Working TBE buffer (0.5x)

The 5x TBE (100 ml) was added to 900 ml of UDW. This solution can be reused three times.

6. Ethidium bromide solution

To prepare stock ethidium bromide solution, a tablet of ethidium bromide (USB Corporation, Cleveland, Ohio, USA) was dissolved in 1 ml of UDW to a concentration of 10 mg/ml. Fifty microliters of the stock solution was then added to 100 ml of the buffer to make of 0.5 μg/ml working concentration. The solution was kept protected from light.

7. Agarose (1%) gel preparation

Agarose (USB Corporation) (0.3 g) was added to 30 ml of either 1x TAE or 1x TBE buffer and dissolved by heating. Molten agarose was allowed to cool down to 50-60°C at 25°C before poring in a gel casting apparatus.

APPENDIX B

Media for *E. coli* cultures

1. LB broth

This medium consists of:

Bacto tryptone	10	g
Bacto yeast extract	5	g
NaCl	5	g

The broth was prepared by completely dissolving all of the above reagents in DW to final volume of one liter. The preparation was sterilized by autoclaving. The broth was stored at 4°C in small aliquots.

2. LB agar plates

This medium consists of:

Bacto tryptone	10	g
Bacto yeast extract	5	g
NaCl	5	g
Agar	15	g

The agar was prepared by dissolving all of the above reagents in DW to final volume of one liter and then sterilized by autoclaving. The solution was poured into petridishes (25 ml/plate), and allowed for solidification at 25°C surface dried then they were stored at 4°C.

3. LB-ampicillin (LB-A) broth (100 μg ampicillin/ml)

The medium was prepared by mixing 400 μl of 250 mg/ml sterilized ampicillin with 1 liter of the autoclaved LB-broth.

4. LB-ampicillin (LB-A) agar (100 μg ampicillin/ml)

One liter of LB agar was prepared, autoclaved, and cooled down to 55°C. Then 400 μl of 250 mg/ml sterilized ampicillin was added to the agar. After gently mixing by swirling, the preparation was poured into petridishes (25 ml/plate). Surface of the agar was dried and the plates were stored at 4°C.

5. LB-ampicillin (100 μg ampicillin/ml)/100 mM IPTG/5% X-Gal agar

Forty microliters of 100 mM IPTG and 40 μl of 5% X-Gal were spread onto the LB-ampicillin agar plate. The agar plate was dried at 37°C before use.

6. LB-kanamycin broth (25 μg kanamycin /ml)

The medium was prepared by mixing 100 μl of 250 mg/ml sterilized kanamycin with 1 liter of the autoclaved LB-broth.

7. LB- ampicillin-kanamycin broth (100 μg ampicillin/+ 25 μg kanamycin /ml)

The medium was prepared by mixing 400 μl of 250 mg/ml sterilized ampicillin and 100 μl of 250 mg/ml sterilized kanamycin with 1 liter of the autoclaved LB-broth.

8. LB- ampicillin-kanamycin agar (100 μg ampicillin/ml, 25 μg kanamycin /ml)

One liter of LB agar was prepared, autoclaved and cooled down to 55°C. Then 400 μl of 250 mg/ml sterilized ampicillin and 100 μl of 250 mg/ml sterilized kanamycin was added to the agar. After gently mixing by swirling, the preparation was poured into petridishes (25 ml/plate). The surface of each agar plate was dried and the plates were stored at 4°C.

9. YT medium (2x)

This medium consists of:

Bacto tryptone	17	g
Bacto yeast extract	10	g
NaCl	5	g

The broth was prepared by completely dissolving all of the above reagents in DW to final volume of one liter. The preparation was sterilized by autoclaving. The broth was stored at 4°C in small aliquots.

10. YT-AG medium (containing 100 μg ampicillin/ml and 2% gluclose 2x-YT-AG)

Four hundred microliters of 250 mg/ml ampicillin and 55.55 ml of 2 M glucose were added to mix with 944.5 ml of warm the autoclaved 2x YT medium.

11. YT agar (2x)

This medium consists of:

Bacto tryptone	17	g
Bacto yeast extract	10	g
NaCl	5	g
Agar	15	g

The agar was prepared by dissolving all of the above reagents in DW to final volume of one liter and then sterilized by autoclaving. The solution was poured into petridishes (25 ml/plate), allowed for solidification at 25°C, Surface dried, and stored at 4°C.

12. YT-AG agar (containing 100 μg ampicillin/ml and 2% glucrose 2x YT-AG)

Four hundred microliters of 250 mg/ml ampicillin and 55.55 ml of 2 M glucose were added into 944.5 ml of warm the autoclaved 2x YT-AG agar. After gently mixing by swirling, the preparation was poured into petridishes (25 ml/plate). The plates were stored at 4°C.

13. 2x YT-AI medium (containing 100 μg ampicillin/ml and 1 mM IPTG)

Four hundred microliters of 250 mg/ml ampicillin and 1 ml of 1 M IPTG were added into one liter of autoclaved 2x YTmedium.

APPENDIX C

Reagents for preparation of competent *E.coli* cells

1. $MgCl_2$ (100 mM)

The solution was prepared by dissolving 20.33 g of $MgCl_2$ in 1,000 ml of DW and sterilized by filtration through a sterile 0.2 μm membrane.

2. $CaCl_2$ (100 mM)

The solution was prepared by dissolving 14.7 g of $CaCl_2$ in 1000 ml of DW and sterilized by filtering though a sterile 0.2 μm membrane by filtration (0.2 μm)

APPENDIX D

Reagents for plasmid DNA extraction

1. Solution I (50 mM glucose, 10 mM EDTA in 25 mM Tris-HCl, pH 8.0)

To prepare solution I, 0.9 g of glucose, 0.33 g of Tris and 0.37 g of EDTA were dissolved in 80 ml of UDW and the pH was then adjusted to 8.0 with 1 M HCl or 1 N NaOH. The solution was sterilized by autoclaving and then stored at 25°C.

2. Solution II (0.2 M NaOH, 1% SDS)

This solution was prepared by mixing 0.2 ml of 5 N NaOH and 0.5 ml of 10% SDS in 4.3 ml of UDW. This solution was prepared immediately before use.

3. Solution III (3 M potassium acetate, pH 5.2)

The solution was prepared by dissolving 29.44 g of potassium acetate in 80 ml of DW. The pH was adjusted to 5.2 with glacial acetic acid. The volume was then made up to 100 ml with UDW before autoclaving, then stored at 25°C.

APPENDIX E

Reagents, solutions, and buffers for recombinant protein purification

1. Lysis buffer-1

To prepare solution, 6.9 g of NaH_2P0_4, 17.54 g of NaCl and 0.68 g of imidazole were dissolved in 800 ml of UDW and the pH was then adjusted to 8.0 with 1 N NaOH. The volume was then made up to 1,000 ml with UDW. The solution was sterilized by filtration through a sterile 0.2 μm membrane and then stored at 25°C.

2. Lysis buffer-2

The solution consists of

NaH_2P0_4	6.9	g
Tris	0.6	g
Urea	480.5	g

The solution was prepared by dissolving all of the above reagents in DW to final volume of one liter and and the pH was then adjusted to 8.0 with 1 N NaOH. The preparation was sterilized by filtration through a 0.2 μm membrane.

3. Washing buffer-1

To prepare this buffer, 6.9 g of NaH_2P0_4, 17.54 g of NaCl and 1.36 g of imidazole were dissolved in 800 ml of UDW and the pH of the solution was then adjusted to 8.0 with 1 N NaOH. The solution was sterilized by filtration through a 0.2 μm membrane.

4. Washing buffer-2

The solution consists of

NaH_2P0_4	13.3	g
Tris	1.2	g
Urea	480.5	g

The solution was prepared by dissolving all of the above reagents in DW to final volume of one liter and the pH was then adjusted to 6.3 with 1 M HCl. The solution was sterilized by though a sterile 0.2 μm membrane filtration as in (3) above.

5. Imidazole in washing buffer-1 (1M)

Imidazole (6.8 g) was dissolved in 90 ml of wash buffer-1 and the pH was adjusted to 8.0 with 1N NaOH. The volume was then made up to 100 ml with UDW. The solution was sterilized by filtration though and then stored at 25°C.

6. Imidazole in lysis buffer-2 (1M)

Imidazole (6.8 g) was dissolved in 90 ml of denaturing wash buffer-2 and the pH was adjusted to 8.0 with 1N NaOH. The volume was then made up to 100 ml with UDW. The solution was sterilized by filtration though a sterile 0.2 μm membrane and then stored at 25°C.

7. Elution buffers pH. 5.9

The solution consists of:

NaH_2P0_4	13.3	g
Tris	1.2	g
Urea	480.5	g

The solution was prepared by dissolving all of the above reagents in DW to final volume of one liter and the pH was then adjusted to 5.9 with 1 M HCl or 1 N NaOH. The preparation was sterilized by filtration (0.2 μm).

8. Elution buffer pH 4.5

The solution consists of:

NaH_2P0_4	13.3	g
Tris	1.2	g
Urea	480.5	g

The solution was prepared by dissolving all of the above reagents in DW to final volume of one liter and the pH was then adjusted to 4.5 with 1 M HCl or 1 N NaOH. The preparation was sterilized by filtration through a 0.2 μm membrane.

9. Elution buffers for proteins with natural conformation

The native elution buffers for proteins with natural conformation contained various concentrations of imidazole in native wash buffer-1. They were prepared by mixing the following components:

	1 M imidazole in wash buffer-1 (μl)	Wash buffer-1 (ml)
Elution buffer 1 (50 mM imidazole):	150	2.85
Elution buffer 2 (100 mM imidazole):	300	2.70
Elution buffer 3 (150 mM imidazole):	450	2.55
Elution buffer 4 (200 mM imidazole):	600	2.40
Elution buffer 5 (250 mM imidazole):	750	2.25
Elution buffer 6 (300 mM imidazole):	900	2.10
Elution buffer 7 (350 mM imidazole):	1,050	1.95
Elution buffer 8 (400 mM imidazole):	1,200	1.80
Elution buffer 9 (450 mM imidazole):	1,350	1.65
Elution buffer 10 (500 mM imidazole):	1,500	1.50

10. Elution buffers for proteins with for proteins with denatured conformation

The buffers for proteins is denatured conformation contained various concentrations of imidazole in denaturing wash buffer. They were prepared by mixing the following components:

	1 M imidazole in wash buffer-2 (μl)	Wash buffer-2 (ml)
Elution buffer 1 (50 mM imidazole):	150	2.85
Elution buffer 2 (100 mM imidazole):	300	2.70
Elution buffer 3 (150 mM imidazole):	450	2.55
	1 M imidazole in wash buffer-2 (μl)	wash buffer-2 (ml)
Elution buffer 4 (200 mM imidazole):	600	2.40
Elution buffer 5 (250 mM imidazole):	750	2.25
Elution buffer 6 (300 mM imidazole):	900	2.10
Elution buffer 7 (350 mM imidazole):	1,050	1.95
Elution buffer 8 (400 mM imidazole):	1,200	1.80
Elution buffer 9 (450 mM imidazole):	1,350	1.65
Elution buffer 10 (500 mM imidazole):	1,500	1.50

APPENDIX F

Reagents for SDS-PAGE

1. Sample buffer (SDS reducing buffer)

The sample buffer was prepared as a stock solution by combination the following ingredients:

Tris-HCl, pH 6.8(0.5 M)	1.0	ml
Glycerol	2.0	ml
SDS (10% solution)	1.6	ml
Bromophenol blue(0.05%)	0.2	ml
2-6-mercaptoethanol	0.4	ml
UDW	2.8	ml

This mixture was stored at 25°C in small aliquots. One part of sample was diluted with equal part of the sample buffer and heated at 100°C for 4 minutes before loading into gel.

2. Tris-HCl (1.5 M, pH 8.8)

To prepare this solution, 18.15 g of Tris base (hydroxymethyl) amino-methane (USB Corporation, USA) was dissolved in 50 ml of UDW, then the pH was adjusted to 8.8 with 1 N HCl. The final volume was brought up to 100 ml with UDW. The solution was filtered through a sterile 0.2 μm membrane. This stock solution was stored at 4°C until use for preparing a working solution.

3. Tris-HCl (0.5 M, pH 6.8)

To prepare this solution, 6.05 g of Tris base (hydroxymethyl aminomethane) (USB Corporation, USA) was dissolved in 50 ml of UDW, then the pH was adjusted to 6.8 with 1 N HCl. The final volume was brought up to 100 ml with UDW. The solution was filtered through a sterile 0.2 μm membrane. This stock solution was stored at 4°C.

4. Sodium dodecyl sulfate (10% SDS; w/v)

This solution was prepared by dissolving 10 g of SDS (Bio-Rad, USA) in 100 ml of UDW.

5. Ammonium persulfate (10%; w/v)

This solution was prepared just before use by dissolving 50 mg of ammonium persulfate (Bio-Rad, USA) in 0.5 ml of UDW.

6. Separating gel (12%)

Polyacrylamide separating gel (12%) was prepared by mixing the following ingredients together:

Tris-HCl, pH 8.8 (1.5 M)	2.5	ml
SDS solution (10%)	0.1	ml
Acrylamide/Bis(30%), 29:1 ratio solution (Bio-Rad, USA)	4.0	ml
UDW	3.35	ml

The reagents were gently mixed and degassed under a vacuum for at least 5 minutes. The polymerization was initiated by adding 50 µl of the 10% ammonium persulfate (freshly prepared) and 5 µl of TEMED (Bio-Rad, USA). The gel was poured into the casting apparatus, over-layered with UDW, and allowed to polymerize for at least 20 minutes at 25°C.

7. Stacking gel (4%)

The stacking gel (4%) was prepared by mixing the following reagents:

Tris-HCl (0.5 M)	2.5	ml
SDS solution (10%)	0.1	ml
Acrylamide/Bis(30%), 29:1 ratio solution (Bio-Rad, USA)	1.3	ml
UDW	6.0	ml

All reagents were mixed gently and degassed under a vacuum for 15 minutes, then 50 µl of freshly prepared 10% ammonium persulfate and 10 µl of TEMED were subsequently added, respectively. After complete mixing and degassing, the upper portion of the gel polymerized in the casting apparatus was rinsed with UDW, the comb was inserted between the glass plates over the polymerized separating gel. The stacking gel was poured and allowed to polymerize for at least 45 minutes at 25°C before use.

9. Electrode (running) buffer (pH 8.3; 5x)

The buffer contained the following reagents: 15 g of Tris base (hydroxy-methyl) aminomethane (Sigma Chemical Co.); 72 g of glycine, and 5 g of SDS. The buffer was prepared by dissolving all of the above reagents in a volume of UDW. After all ingredients were dissolved, the volume was made up to one liter with UDW. The buffer was stored at 4°C until use for preparing a working electrode (running) buffer.

10. Working electrode (running) buffer (1x)

Sixty ml of the 5x electrode buffer (Section 9) was diluted with 240 ml of UDW. Each preparation of the working running buffer was used for only one electrophoretic run.

11. Preparation of solutions for Colloidal Coomassie Brilliant Blue stain

11.1 Fixing solution

The solution was freshly prepared by mixing 10 ml of 85% *o*-phosphoric acid and 20 ml of methanol. The final volume was made to 100 ml with deionized distilled water.

11.2 Stock staining solution A

The solution was prepared by dissolving 4 g of ammonium sulfate in 20 ml of deionized distilled water then 0.95 ml of 85% *o*-phosphoric acid was added. The final volume was made to 40 ml with deionized distilled water.

11.3 Stock staining solution B

The solution was prepared by dissolving 0.5 g of Coomassie Brillient Blue G-250 in 1 ml of deionized distilled water.

11.4 Staining solution, freshly prepared:

The solution was prepared by mixing 1 ml of stock staining solution B with 40 ml of stock staining solution A. Then 10 ml of methanol was added and mixed.

11.5 Neutralization solution

The solution was prepared by dissolving 6 g of Tris-base in 250 ml of deionized distilled water. The pH was adjusted to 6.5 with *o*-phosphoric acid and the final volume was brought up to 500 ml with deionized distilled water.

11.6 Washing solution

The solution was prepared by adding 125 ml of methanol in 375 ml of deionized distilled water.

11.7 Stabilizing solution

The solution was prepared by dissolving 100 g of ammonium sulfate in 250 ml of deionized distilled water. The final volume was brought up to 500 ml with deionized distilled water.

APPENDIX G

Reagents for Western blot analysis

1. Transfer buffer (blotting buffer, pH 8.3) (25 mM Tris, 192 mM glycine, and 20% [v/v] methanol)

To prepare 4,000 ml of this buffer, 12.12 g of Tris base (hydroxymethyl aminomethane) and 57.60 g of glycine was dissolved in 3,200 ml of UDW. Subsequently, 800 ml of methanol was added to yield 20% (v/v).

2. Phosphate buffered saline (0.01 M PBS, pH 7.4)

The solution was prepared by dissolving 1.22 g of anhydrous Na_2HPO_4, 0.17 g of anhydrous NaH_2PO_4 and 8.77 g of NaCl in 1 liter of DW. The pH of this solution was adjusted to 7.4 with 1 N HCl.

3. Tris buffer (0.15 M Tris-HCl, pH 9.6)

The buffer was prepared by dissolving 18.15 g of Tris-base in 500 ml of deionized distilled water. The pH of this solution was adjusted to 9.6 with 1 N HCl. The final volume was brought up to 1,000 ml with distilled water.

4. Phosphate buffer (1/15 M PB, pH 7.6)

The buffer was prepared by dissolving 0.06 g of NaH_2PO_4 and 0.47 g of Na_2HPO_4 in 57.7 ml of UDW. The pH of this solution was adjusted to 7.6 with 1 N HCl.

5. Washing buffer (0.05% Tween-20 in PBS, pH 7.6; PBS-T)

This solution was prepared by adding 0.5 ml of Tween-20 in one liter of 0.01 M PBS (pH 7.4) and mixed.

6. Blocking solution (3% BSA in PBS, pH 7.6)

The solution was prepared by dissolving 3 g of bovine serum albumin (BSA, Sigma Chemical Co.) and 0.5 g of gelatin (Sigma Chemical Co.) in 100 ml of 0.01 M TBS, pH 7.4.

7. Diluent solution (0.2% BSA in PBS, pH 7.6)

The solution was prepared by dissolving 0.2 g of BSA in 100 ml of 0.01 M PBS, pH 7.6.

8. Conjugate solution

This solution was prepared by diluting goat anti-human immunoglobulins-AP conjugate (Southern) or goat anti-human immunoglobulins-HP conjugate (Southern) with diluent solution to make the desired.

9. Substrate solution

9.1 Commercial BCIP/NBT substrate solution was purchased from Kierkegaard & Perry Laboratory (KPL), USA. To prepare this solution, one part of concentrate substrate was diluted with three part of diluent (0.15 M Tris-HCl, pH 9.6). This substrate was prepared freshly before use and always protected from the light.

9.2 The solution was freshly prepared by dissolving 0.02 g of 2, 6-dichlorophenolindophenol (Sigma Chemical Co.) in 10 ml of 1/15 M phosphate buffer, pH 7.6. Ten microliters of 30% H_2O_2 was added immediately before use.

APPENDIX H

Reagents for phage bio-panning

1. Coating buffer (carbonate-bicarbonate buffer, pH 9.6)

The buffer was prepared by dissolving 1.26 g of $NaHCO_3$ in 300 ml of DW, then the pH was adjusted to 9.6 with 0.05 Na_2CO_3 (0.53 g in 100 ml of DW).

2. Phosphate buffered saline (0.01 M PBS, pH 7.4)

The solution was prepared by dissolving 1.22 g of anhydrous Na_2HPO_4, 0.17 g of anhydrous NaH_2PO_4 and 8.77 g of NaCl in 1 liter of DW. The pH of this solution was adjusted to 7.4 with 1 N HCl.

3. Washing solution (0.1% PBS-T)

Washing solution (PBS-T) was prepared by mixing 1 ml of Tween-20 (USB, OH, USA) in 0.01M PBS, pH 7.4 to a 0.1 % concentration.

4. Blocking solution

The solution was prepared by dissolving 5 g of skim milk (HIMEDIA, Mumbai, India) in 100 ml of 0.01 M PBS, pH 7.4.

5. Glycine-HCl (0.1 M, pH 2.2)

This solution was prepared by dissolving 7.51 g of glycine in 900 ml of DW. The pH of this solution was adjusted to 2.2 with 1 N HCl. The final volume was brought up to 1,000 ml with DW.

APPENDIX I

Reagents for indirect ELISA HuScFv-ELISA

1. Coating buffer (carbonate-bicarbonate buffer, pH 9.6)

The buffer was prepared as previously described.

2. Phosphate buffered saline (0.01 M PBS, pH 7.4)

The solution was prepared as previously described.

3. Washing solution (0.05% PBS-T)

Washing solution (PBS-T) was prepared.

4. Blocking solution

The solution was prepared.

5. Conjugate solution

This solution was prepared by diluting goat anti-human immunoglobulins-HRP conjugate (Southern) with 0.05% PBS-T to make the desired dilution.

6. Diluents solution

The diluents solution was prepared by dissolving 0.2 g of BSA and 0.2 g of gelatin in 100 ml of 0.01 M PBS, pH 7.4.

7. Substrate buffer (0.1 M citrate buffer, pH 4.5)

The buffer was prepared by dissolving 14.7 g of trisodium citrate ($Na_3C_6H_5O_7.H_2O$) in DW. The volume was made up to 500 ml after the pH was adjusted to 4.5 with 1M HCl.

8. Substrate solution

The substrate solution consisted of 0.05% 1, 4-*p*-phenylenediamine-dihydrochloride (PPD) (Sigma Chemical Co.) in citrate buffer, pH 4.5 and 0.01% of 30% H_2O_2. This solution was prepared freshly before use and always protected from light.

9. Stop solution (1N NaOH)

The solution was prepared by dissolving 20 g of NaOH in 500 ml of UDW.