APPENDIX

APPENDIX A

CHEMICALS AND EQUIPMENTS

1. CHEMICALS AND ANTIBODIES

All chemicals used in this study were analytical grade reagents

- Acrylamide (BDH Laboratory Supplies, UK)
- Agarose (electrophoresis grade) (Sigma-Aldrich Co., USA)
- Ampicillin (Sigma-Aldrich Co.,USA)
- Bis-acrylamide (BDH Laboratory Supplies, UK)
- Developer and replenisher (Kodak, NY, USA)
- DMEM (Gibco, Grand Island, NY, USA)
- Ethanol (Merck, Darmstadt, Germany)
- Ethidium bromide (Sigma-Aldrich Co.,USA)
- Fetal calf serum (Gibco, Grand Island, NY, USA)
- Glacial acetic acid (BDH Laboratory Supplies, UK)
- Glycreol (Sigma-Aldrich Co., USA)
- HCl (Merck, Darmstadt, Germany)
- HRP-conjugated anti-HA (Roache, IN, USA)
- HRP-conjugated goat anti-mouse Igs antibody (Amersham-Pharmacia Biotech, Buckinghamshire, UK)
- Kanamycin (Sigma-Aldrich Co., USA)

- Methanol (Merck, Darmstadt, Germany)
- MOPS (Amersco, USA)
- NaCl (BDH Laboratory Supplies, UK)
- NaOH (BDH Laboratory Supplies, UK)
- Polyvinylidene-fluoride membrane (PALL, East Hill, NY, USA)
- SDS (Sigma-Aldrich Co., USA)
- Skimmed milk (Difco Laboratories, Detroit, MI, USA)
- TEMED (Sigma-Aldrich Co., USA)
- Tris Base (Sigma-Aldrich Co., USA)
- Tripsin/EDTA (Gibco, Grand Island, NY, USA)
- Tryptone (Life Technologies, Scotland)
- Tween 20 (Fluka, Buchs, Switzerland)
- Yeast extract (Life Technologies, Scotland)
- 2-propanol (Merck, Darmstadt, Germany)

2. MOLECULAR REAGENTS AND MATERIALS

- 1 kb DNA and protein marker (Fermentus, MA, USA)
- Restriction enzymes (*Sal*I, *Not*I, *Pac*I, *Pme*I) (Fermentus, MA, USA)
- dNTPs (Fermentus, MA,USA)
- T4 DNA ligase (Fermentus, MA, USA)
- KOD DNA polymerase (Novagen, Nottingham, UK)
- QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany)
- QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany)

- QIAGEN Plasmid Midi Kit (QIAGEN, Hilden, Germany)
- QuikChange^{TM}Site-direct Mutagenasis Kit (Stratagene, CA, USA)
- Taq DNA polymerase (Eppendrof, USA)
- TransFectin^{TM} Lipid Reagent (BIO-RAD Laboratories, USA)

3. MICROORGANISMS

- *Escherichia coli* Nova Blue (Novagen, Madison, WI, USA)
- *Escherichia coli* BJ5183 (Stratagene, CA, USA)
- *Escherichia coli* DH10B (Stratagene, CA, USA)

4. EQUIPMENTS

- 37 °C incubator (JP Selecta, Barcelona, Spain)
- Electrophoretic power supply, ECPS 3000/150 (Amersham Phamacia Biotech, Sweden)
- Microcentrifuge (Eppendorf AG, Hamburg, Germany)
- Microplate Reader EL340 (BIO-TEK Instruments, USA)
- MRX-150 refrigerated microcentrifuge (TOMY, Japan)
- PCR Mastercycler personal (Eppendrof, USA)
- RT6000 D refrigerated centrifuge (Sorvall, USA)
- UV160 Spectrophotometer (Shimadzu, Japan)
- Gel-Doc Transilluminator (BIO-RAD Laboratories, USA)

APPENDIX B

REAGENT PREPARATIONS

1. 1% agarose gel

Agarose gel	0.5	gm
1X TAE buffer until the volume	50	ml

2. Ethidium bromide working solution (10 mg/ml)

Ethidium bromide	1.0	gm
DW until the volume	100	ml

Keep in the dark bottle and store at room temperature.

3. 6X gel loading buffer

Bromphenol blue	0.25	%
Glycerol	30	%

Mix thoroughly and keep at -20 °C.

4. 10X Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris-base	48.40	gm
Glacial acetic acid	11.42	ml
0.5 M EDTA (pH 8.0)	20	ml

Dissolve all ingredients in distilled water (DW) and fill up to 1,000 ml and keep at 4 °C.

5. Reagents for using in ELISA

5.1 0.05% Tween20 in PBS (Washing buffer)

10 mM PBS	500	ml
Tween 20	0.25	ml

Mix well and store at room temperature.

5.2 10 mM Phosphate buffer saline (PBS), pH 7.2

NaCl	8.00	gm
KCl	0.20	gm
Na_2PO_4	1.15	gm
KH_2PO_4	0.20	gm

Dissolve all ingredients in DW until the volume 900 ml.

Adjust the pH to 7.2 with 1 N NaOH.

Add DW until the volume was reached 1000 ml and store at 4.

6. Reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

6.1 1.5 M Tris-HCl pH 8.8

Tris-base	18.15	gm
Deionized DW	75	ml

Adjusted the pH to 8.8 with concentrated HCl.

Adjusted the volume to 100 ml with deionized DW and stored at 4 °C

6.2 0.5 M Tris-HCl pH 6.8

Tris-base	6.0	gm
Deionized DW	80	ml

Adjusted the pH to 6.8 with concentrated HCl.

Adjusted the volume to 100 ml with deionized DW and stored at 4 °C

6.3 Running buffer

Tris-base	1.51	gm
Glycine	7.20	gm
Sodium dodesyl sulphate	0.50	gm

Dissolved in 500 ml DW and kept at 4 °C

6.4 Blotting buffer

Tris-base	3.30	gm
Glycine	14.41	gm
Sodium dodesyl sulphate	0.50	gm
Dissolved all gradients with DW	700	ml
Methanol	200	ml

Dissolved all ingradients in DW and filled up to 1,000 ml and kept at 4 °C

6.5 Copolymerization of 4% stacking gel (5 ml)

Stock acrylamide 30%	0.83	ml
0.5 M Tris-HCl pH 6.8	0.63	ml
10% SDS	0.05	ml
DW	3.40	ml
10% Ammonium persulfate	0.05	ml
TEMED	0.01	ml

6.6 Copolymerization of 12% Separating gel (15 ml)

Stock acrylamide 30%	6.0	ml

1.5 M Tris-HCl pH 8.8	3.8	ml
10% SDS	0.15	ml
DW	5.0	ml
10% Ammonium persulfate	0.15	ml
TEMED	0.01	ml

7. Reagents for using in plasmid mini-preparation

7.1 3 M Sodium Acetate pH 7.0

$NaAcet \cdot 3H_2O$	40.8	gm

Adjust pH to 7.0 with NaOH/HCl

Dissolve in 100 ml DW and keep at 4 °C

7.2 Potassium Acetate

Potassium Acetate	29.4	gm
Glacial acetic acid	11.5	ml

Dissolve in 100 ml DW and keep at 4 °C.

7.3 10 M NaOH

NaOH	200	gm

Dissolve in 500 ml DW and keep at 4 °C.

7.4 10% SDS

SDS	5	gm

Dissolve in 50 ml DW and store at room temperature.

7.5 7.5 M NH_4 Acetate

NH_4 Acetate	57.8	gm

Dissolve in 100 ml DW and keep at 4 °C.

7.6 1 M glucose buffer

D-glucose	18.02	gm

Dissolve all ingredients in DW and fill up to 100 ml. Autoclave and keep at 4 °C.

7.7 0.5 M EDTA pH 8.0

EDTA	37.22	gm
DW	100	ml

Adjust pH to 8.0, add DW to 200 ml and keep at 4 °C.

7.8 10X GLUCOMIX

1 M glucose buffer	50	ml

0.5 M EDTA pH 8.0	20	ml
1 M Tris pH 8.0	25	ml
DW	5	ml

Autoclave and keep at 4 °C.

7.9 1X glucomix-lysozyme solution

10X GLUCOMIX	300	µl
Lysozyme stock (50mg/ml in DW)	300	µl
DW	2.4	ml

Keep on ice or store at 4 °C for 7 days.

8. Media for bacterial culture

8.1 LB broth

Tryptone	10	gm
Yeast extract	5	gm
Sodium Chloride	10	gm

Dissolve all ingredients in 1,000 ml distilled water.

Sterile by autoclave and keep at 4 °C.

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