VIII. APPENDIX

I. Reagents for production of monoclonal antibodie

1. Phosphate buffered saline (PBS) pH 7.2

NaCl	8.00	gm
KCl	0.20	gm
$Na_2 HPO_4$	1.15	gm
$KH_2 PO_4$	0.20	gm

- Added distilled water to about 900 ml and stirred on magnetic stirrer until well dissolved.
- Adjusted the pH to 7.2 with 1N HCl or 1N NaOH.
- Filled up to 1000 ml with distilled water and stored at room temperature.

2. Iscove's Modified Dulbecco's Medium (IMDM)
- Add IMDM powder to distilled water with gentle stirring.
- Rinsed out inside of package to remove all traces of powder.
- Added 3.02 gm of $NaHCO_3$.
- Added gentamicin and amphotericin B to final concentration of 40 μg/ml and 2.5 μg/ml, respectively.
- Added distilled water to about 900 ml, mixed well and adjusted pH to 7.4 with 1M NaOH or 1N HCl.
- Filled up with distilled water to 1000 ml.
- Sterilized by filtration and stored at 4°C.

3. 10% FCS-IMDM
- Mixed 10 ml of heat inactivated fetal calf serum and 90 ml IMDM together by sterile technique in a laminar air flow and kept at 4°C.

4. 0.2% trypan blue in normal saline

Trypan blue powder	0.20	gm
Normal saline solution	100	ml

- Dissolved trypan blue in normal saline solution and stored at room temperature.

5. Lysing solution (Hypotonic NH_4Cl)

NH_4Cl	8.29	gm
$KHCO_3$	1.00	gm
disodium EDTA	0.04	gm

- Added distilled water to about 900 ml and mixed until well dissolved.
- Adjusted pH to 7.4 with 1N HCl.
- filled up to 1000 ml with distilled water and stored at 4°C.

6. HAT medium

10% FCS-IMDM	99	ml
10 mM Hypoxanthine, 50 μM Aminopterin, 1.6 mM Thymidine (HAT) 100X stock	1	ml
0.6% of 2-Mercaptoethanol	60	μl

- Mixed all ingradients together until well dissolved.
- Sterilized by filtration and stored at 4°C.

7. HT medium

10% FCS-IMDM	99	ml
10 mM Hypoxanthine, 1.6 mM Thymidine (HT) 100X stock	1	ml
0.6% of 2-Mercaptoethanol	60	μl

- Mixed all ingradients together until well dissolved.
- Sterilized by filtration and stored at 4°C.

II. Regents for isolation of human blood cells and cell culture

1. RPMI medium
   - Added RPMI 1640 medium powder to distilled water with gentle stirring.
   - Rinsed out inside of package to remove all traces of powder.
   - Added 2.0 gm of $NaHCO_3$.
   - Added gentamicin and amphotericin B to final concentration of 40 μg/ml and 2.5 μg/ml, respectively.
   - Added distilled water to about 900 ml, mixed well and adjusted pH to 7.2 with 10% acetic acid.
   - Filled up with distilled water to 1000 ml.
   - Sterilized by filtration and stored at 4°C.

2. 10% FCS-RPMI 1640
   - Mixed 10 ml of heat inactivated fetal calf serum and 90 ml RPMI-1640 together by sterile technique in a laminar air flow and kept at 4°C.

III. Reagents for indirect immunofluorescent technique

1. 1% bovine serum albumin in phosphate buffered saline pH 7.2 with 0.02% azide (1% BSA-PBS azide)

Bovine serum albumin fraction IV	1.00	gm
Phosphate buffered saline (PBS) pH 7.2	80	ml

   - Mixed until well dissolved and added 10% sodium azide to make 0.02% concentration, finally top up to 100 ml with PBS.
   - Stored at 4°C.

IV. Reagents for Flow cytometrical analysis

1. Fixative in PBS

Paraformaldehyde	0.5	gm
PBS pH 7.2	100	ml

   - Dissolved Paraformaldehyde in 60 °C PBS pH 7.2 by stirring on magnetic plate.
   - Remove the particle by filtration and stored at room temperature.

V. Reagents for preparation M6 DNA

A. Reagents for preparation of competent bacteria

1. PSI A medium

Bacto yeast extract	5.00	gm
Bacto tryptone	20.00	gm
$MgSO_4$	5.00	gm
Bactoagar	21.00	gm

   - Added distilled water to about 900 ml, mix well.
   - adjusted pH to 7.6 with KOH.
   - Filled up with distilled water to 1000 ml.
   - Sterilized by autoclaving and pour into 10 cm plates (25 ml/ plate).
   - Stored at 4°C.

2. PSI B broth
   - PSI A medium without agar.
   - Stored at 4°C.

3. TFB I

KoAc (MW 98.14)	0.588	gm
$RbCl_2$ (MW 120.9)	2.42	gm
$CaCl_2 .2H_2O$(MW 147.0)	0.294	gm
$MnCl_2$ (MW 197.9)	1.98	gm
Glycerol	30	ml

   - Added distilled water to about 180 ml and mixed well.
   - Adjusted the pH to 5.8 with 0.2M HAc
   - Filled up with distilled water to 200 ml.
   - Sterilized by filtration and stored at room temperature.

4. TFB II

3-(N-morpholino)propanesulfonic acid (MOPS) MW 209.3	0.42	gm
$CaCl_2 . 2 H_2O$ (MW 147.0)	2.2	gm
$RbCl_2$ (MW 120.9)	0.242	gm
Glycerol	30	ml

   - Added distilled water to about 180 ml, mix well.
   - Adjusted pH to 6.5 with 5M KOH.
   - Filled up with distilled water to 200 ml.
   - Sterilized by filtration and stored at room temperature.

B. Reagents for transformation of competent bacteria by plasmid DNA

1. LB broth

Bacto tryptone	10.00	g m
Bacto yeast extract	5.00	gm
NaCl	10.00	gm

   - Added distilled water to about 900 ml, mix well.
   - Adjusted pH to 7.0 with 5 N NaOH.
   - Filled up with distilled water to 1000 ml.
   - Sterilized by autoclaving and stored at room temperature.

2. LB antibiotic plate

LB broth	1000	ml
Bacto agar	21.00	gm

- Sterilized by autoclaving and left it cool down to 56 °C.
- Added ampicillin and tetracycline to final concentration of 15 μg/ml and 10 μg/ml, respectively.
- Mix well and pour into 10 cm plates (25 ml/plate).
- Stored at 4°C.

C. Reagents for preparation of plasmid DNA

1. 0.5 M EDTA (pH 8.0)

EDTA (MW 372.24)	18.61	gm

- Added distilled water to about 90 ml.
- Adjusted pH to 8.0 with 5 N KOH.
- Filled up with distilled water to 100 ml.
- Sterilized by autoclaving and stored at room temperature.

2. 1 M Tris HCl (pH 8.0)

Tris base (MW 121.1)	12.11	gm

- Added distilled water to about 90 ml.
- Mix well
- Adjusted pH to 8.0 with 8 N HCl.
- Filled up with distilled water to 100 ml
- Sterilized by autoclaving and stored at room temperature.

3. 1 M Glucose

D-glucose (MW 180.16)	18.016	gm

- Added distilled water 100 ml, mix well.
- Sterilized by filtration and stored at 4°C.

4. 10X Glucomix

1 M Glucose	50	ml
0.5 M EDTA (pH 8.0)	20	ml
1 M Tris HCl (pH 8.0)	25	ml

- Added distilled water 5 ml, mix well.
- Sterilized by filtration and stored at 4°C.

5. 1X Glucomix-lysozyme

10 X Glucomix	300	µl
lysozyme (50 mg/ml in distilled water)	300	µl

- Added distilled water 2.4 ml, mix well.
- Place on ice bath, used immediately after preparation.

6. 10% SDS solution

Sodium dodecyl sulfate (MW 288.34)	10.00	gm
Distilled water	30	ml

- Mix well by magnetic stirrer on hot plate at 50°C.
- Added distilled water to 100 ml.
- Sterilized by filtration and stored at room temperature.

7. 5M Potassium acetate

Potassium acetate (MW 98.15)	49.07	gm

- Added distilled water 100 ml, mix well.
- Sterilized by filtration and stored at 4°C.

8. Potassium acetate solution

5M Potassium acetate	60	ml
Glacial acetic acid	11.5	ml

- Added distilled water 28.5 ml, mix well.
- Sterilized by filtration and stored at room temperature.

9. RNase-H_2O

RNase Solution (Stock Conc 10 mg/ml)	10	µl

- Added distilled water 1 ml, mix well.
- Used immediately after preparation.

10. Saturated diethylether

Diethylether	50	ml
distilled water	25	ml

- Mix well, left until it separated into 2 layers (used upper layer)
- Stored at room temperature.

11. 1% SDS in 0.2 N NaOH

1 M NaOH	20	ml
10% SDS	10	ml
distilled water	70	ml

- mix well, stored at room temperature.
- Used within 7 days after preparation.

12. TB (Terrific broth)-antibiotic broth
preparation 2 ingredients as follows:

A. Bacto tryptone	10.00	gm
Bacto yeast extract	24.00	gm
Glycerol	4	ml
distilled water	900	ml
B. KH_2PO_4 (MW 136.09)	2.31	gm
K_2HPO_4 (MW 174.18)	12.54	gm
distilled water	100	ml

- Sterilized both A and B gradient separately by autoclaving.
- Mix this 2 part together, leave it cool down about 60 °C or slightly lower.
- Added ampicillin and tetracycline to final concentration of 15 µg/ml and 10 µg/ml, respectively.
- Mix well and stored at 4°C.

13. 10 mM Tris pH 7.5, 1 mM EDTA ($T_{10}E_1$)

1 M Tris pH 8.0	1	ml
0.5 M EDTA pH 8.0	200	µl

- Added distilled water to about 90 ml, mix well.
- Adjusted pH to 7.5 with 8M HCl.
- Filled up with distilled water to 100 ml.
- Mix well and stored at room temperature.

14. Ethidium bromide (10 mg/ml)

Ethidium bromide	1.00	gm
distilled water	100	ml

- Mix well by shaking about 1 hour.
- Stored in dark place at room temperature.

V. Reagents for DEAE-dextran transfection

1. Minimum Essential Medium (MEM)
   - Added MEM powder to distilled water with gentle stirring.
   - Rinsed out inside of package to remove all traces of powder.
   - Added 2.2 gm of $NaHCO_3$.
   - Added gentamicin and amphotericin B to final concentration of 40 µg/ml and 2.5 µg/ml, respectively.
   - Added distilled water to about 900 ml, mixed well and adjusted pH to 7.2 with 10% acetic acid.
   - Filled up with distilled water to 1000 ml.
   - Sterilized by filtration and stored at 4°C.

2. 5% FCS-MEM medium

Fetal calf serum	5	ml
MEM	95	ml

   - Mix well, and stored at 4°C.

3. 0.5 mM EDTA-PBS

PBS pH 7.2	100	ml
0.5 M EDTA pH 8.0	100	µl

   - Mix well.
   - Sterilized by filtration and stored at room temperature.

4. DEAE-Dextran Stock Solution (10 mg/ml)

DEAE-Dextran (MW 500,000)	0.10	gm
PBS pH 7.2	10	ml

   - Mix well.
   - Sterilized by filtration.
   - Aliquot to vials (500 µl/vial).
   - Store at -20 °C.

5. Chloroquine diphosphate Stock Solution (10 mM)

Chloroquine diphosphate (MW 515.9)	0.0519	gm
PBS pH 7.2	10	ml

   - Mix well.
   - Sterilized by filtration.
   - Aliquot to vials (500 µl/vial).
   - Store at -20 °C.

6. 10% DMSO-PBS

PBS pH 7.2	90	ml
Dimethyl sulfoxide	10	ml

- Mix well.
- Sterilized by filtration.
- Stored at room temperature.

VI. Reagents for agarose gel electrophoresis

1. 5X TBE

Tris-base (MW. 121.1)	54.0	gm
Boric-acid (MW. 16.18)	27.5	gm
0.5 M EDTA (pH 8.0)	20	ml

- Added distilled water to 1000 ml.
- Mix well, and stored at room temperature.

2. 1X TBE

5X TBE	40	ml
- Distilled water	160	ml

- Mix well, and stored at room temperature.

3. 1% Agarose

Agarose	0.25	gm
1x TBE	25	ml

- Boil agar, and then left it in water bath at 60 °C before pour it into the electrophoresis chamber.

VII. Reagents for Capture ELISA

1. 3′, 3′, 5′, 5′,-tetramethylbenzidine (TMB) substrate solution

3′, 3′, 5′, 5′,-tetramethylbenzidine (TMB)	.0016	gm
dimethylsulfoxide (DMSO)	500	µl
0.1 M Sodium acetate(pH 6.0)	9.9	ml
Hydrogen peroxide	50	µl

- Dissolved 3′, 3′, 5′, 5′,-tetramethylbenzidine (TMB) in DMSO and mix well.
- Added 0.1 M Sodium acetate (pH 6.0) and stirred on magnetic-

plate until well dissolved.
- Added Hydrogen peroxide and mixed well.
- Used immediately after preparation.

2. Carbonate/bicarbonate coating buffer pH 9.6, 0.1M.

Na_2CO_3	4.24	gm
$NaHCO_3$	5.04	gm

- Dissolved Na_2CO_3 and $NaHCO_3$ in 1000 ml distilled water and check pH.
- Store at 4°C.

3. Phosphate buffered saline with 0.05% Tween (PBST)

Phosphate buffered saline pH 7.2	1000	ml
Tween-20	0.5	ml

- Add Tween 20 into Phosphate buffered saline pH 7.2 and mix well.
-Used within a few days after preparation.

4. 5% bovine serum albumin in phosphate buffered saline pH 7.2 with 0.02% azide (5% BSA-PBS azide)

Bovine serum albumin fraction IV	5.00	gm
Phosphate buffered saline (PBS) pH 7.2	80	ml

- Mixed until well dissolved and added 10% sodium azide to make 0.02% concentration, finally top up to 100 ml with PBS.
- Stored at 4°C.

VIII. Reagents for cell proliferation assay

1. [^{3}H] thymidine 4 μCi per ml

[^{3}H] thymidine (Amersham)	0.120	ml
10% FCS-RPMI	30.0	ml

- Mixed well and stored at 4°C.

2. Liquid scintillation fluid

2,5-diphenyloxazole (PPO)	10.00	gm
Dimethyl POPOP	0.25	gm
Toluene	2.5	L

- Dissolved the two reagents in toluene and stored at room temperature.

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