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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances.

Avidin-biotinylated horseradish peroxidase complexes (DAKOPATTS, Glostrup, Denmark)

Complete Freund 's Adjuvant (Difco , Detroit,MA,USA)

Copper sulphate $CUSO_4$. 5 H_2O (E.Merck,Damstadt, W.Germany)

Disodium hydrogen phosphate (Na_2PO_4) (E.Merck, Damstadt, W.Germany)

Ethanol absolute c_2H_5OH)(E.Merck,Damstadt,W.Germany)

Hydrochloric acid (HCl) (E.Merck,Damstadt,W.Germany)

Hydrogen peroxide (H_2 O_2)(E.Merck ,Damstadt, W.Germany)

Magnesium sulphate ($MgSO_4.7H_2O$) (E.Merck,Damstadt, W.Germany)

Methanol (CH_3OH) (E.Merck,Damstadt,W.Germany)

Nobel agar (Difco,Detroit,MA,USA)

Protein A-sepharose CL-4B (Pharmacia ,Uppasala,Sweden)

Sodium acetate (CH_3COONa) (BDH,England

Sodium azide (NaN_3) (E.Merck,Damstadt,W.Germany)

Sodium bicarbonate ($NaHCO_3$) (BDH,England)

Sodium chloride (NaCl) (E.Merck,Damstadt,W.Germany)

Sodium dihydrogen phosphate (NaH_2Po_4) (E.Merck,Damstadt,W.Germany)

Sodium hydroxide (NaOH) (BDH ,England)

Tris (Hydroxymethyl aminomethane (Tris: $C_4H_{11}No_3$) (E.Merck,Damstadt,W.Germany)

B. Antiserum and serum

Equine anti-rabies globulin,Fluorescein labeled (BBL,MD,USA)

Goat anti-horse IgG, biotinylated (Vector,CA,USA)

Goat anti-rabbit IgG, biotinylated (Sigma,MO,USA)

Goat serum, normal (Sigma,MO,USA)

Rabbit anti-rabies IgG (Difco,Detroit,MA.USA)

Swine anti-rabbit serum (DAKO,Igs.,Glastrup,Denmark)

C. Glassware

Beaker (Pyrex,Corning,N.Y.,USA)

Cylinder (Witeg,W.Germany)

Disposible syringe (Nipro,Tokyo,JAPAN)

Erlenmeyer flask(Pyrex, Corning ,NY,USA)

Disposible 96 wells plate (Costar,

Glass tube (Pyrex,Corning,NY ,USA)

D. Instrument

Analytical balance (Precisa,Switzerland)

Automatic pipette (Gilson,Lyon,France)

Centrifuge

Fraction collector, Model alpha 400 (Buchler Fractometer, USA)

Incubator (Forma Sciencific,Ohio,USA)

Mixer Vortex (Scientific industries,N>Y>,USA)

pH meter,model 10 (Corning,N.Y.,USA)

Refrigerator (westinghouse, USA)

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APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagents for Immunoglobulin G Preparation.

1.1 Phosphate buffer saline 0.05 M,pH 7.4

Stock solution A:

NaH_2Po_4	15.6	g
Distilled water to	1000	ml

Stock solution :

Na_2HPO_4	14.2	g
Distilled water	1000	ml

0.05 M. PBS, pH 7.4

Solution A	250	ml
Solution B	750	ml
NaCl	8.0	g
Distilled water to	2000	ml

Store at 4 C

1.2 Tris-(hydroxymethyl) aminomethane(Tris)buffer 0.05 M. in 0.15 M. NaCl,pH 8.6

$C_4H_{11}NO_3$	6.057	g
NaCl	8.766	g
Distilled water to	1000	ml

Adjust to the pH to 8.6 with 1 N HCl,and store at 4 C

1.3 Acetate buffer 0.05 M in 0.15 M.NaCl,pH4.3

Stock solution A:

$CH_3COONa.3H_2O$	13.6	g

Distilled water to 1000 ml

Stock solution B:

CH_3COOH	5.75	ml
Distilled water to	1000	ml

0.05 M.Acetate buffer ,pH 4.3

Solution A	132	ml
Solution B	368	ml
NaCl	8.766	g
Distilled water to	1000	ml

Store at 4 C

1.4 NaOH 1 N

NaCl	40	g
Distilled water to	1000	ml

1.5 HCl 1 N

HCl	36.5	g
Distilled water to	1000	ml

2. Reagent for dot - immunoblot

2.1 Phosphate buffer saline (PBS) 0.01 M,pH7.4

Na_2HPO_4	2.85	g
NaH_2PO_4 .12 H_2O	7.0	g
NaCl	9.25	g
Distilled water to	1000	ml

Adjust the pH to 7.4 with 1 N NaOH

2.2 Hydrogen peroxide 1% in methanol

Add 35% H_2O_2 0.86 ml into methanol 30 ml

2.3 Avidin-biotin-peroxidase complex (ABC)

Commercially available ABC is prepared by mixing 1 ul of "A"(avidin) and 1 ul of "B" (biotin peroxidase) in PBS,pH 7.4 ml for 30 min. at room temperature before use.

2.4 Substate solution 4-Cholro-1-naphthol (4ClN)

Dissolve 30 mg of 4ClN in 10 ml of Methanol.It could be stored at 4C in a dark bottle for 14 days. For use, 2 ml of 4ClN solution was mixed with 10 ml of PBS and 5 ul of 30% hydrogen peroxide.The substrate must to be used immediately

3. Reagent for immunodiffusion test

3.1 Sodium barbital buffer (0.05 M), pH 8.2

Barbital sodium	47.6	g
1 N HCl	69	ml
10% NaN_3	4.2	ml
Distilled water to	4200	ml

Adjust pH to 8.2

3.2 Protein staining

3.2.1 Protein staining solution

Coomasie brilliant blue R	5.0	g
Distilled water	1000	ml

stir overnight until dissolved.

3.2.2 Destaining solution

Distilled water	1000	ml
CH_3COOH	2000	ml
Methanol	1000	ml

Mix and store at room temperature.

3.2.3 Agar gel 1.5%

Special agar Nobel	1.5	g
Distilled water	100	ml

Heat the agar in a double boiling water until dissolved and aliquot into 20 ml test tube,allow to cool and store in 4 C until use.

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CURRICULUM VITAE

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