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APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical substances

Acetone, AR grade (BDH, England)

Agarose (Kallestad, MN, U.S.A.)

Amphotericin B (Squibb & Sons, Inc., U.S.A.)

Bovine serum albumin (Sigma, MO, U.S.A.)

Citric acid ($\text{H}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$) (Mallinckrodt Inc., MO, U.S.A.)

Disodium hydrogen phosphate (Na_2HPO_4) (E. Merck, Darmstadt, W. Germany)

Evans blue (Sigma, MO, U.S.A.)

EDTA (Ethylenediaminetetraacetic acid) (BDH, England)

Fetal bovine serum (Flow, North Ryde, Australia)

Glycerol ($\text{CH}_2\text{OHCHOHCH}_2\text{OH}$) (Farmitalia Carlo Erba, Milan, Italy)

HEPES (N-2-hydroxyethylpiperazine-N-2ethanesulfonic) (Sigma, MO, U.S.A.)

Minimum Essential Medium (GIBCO, Grand Island, NY, U.S.A.)

O-phenylenediamine (OPD) (Sigma, MO, U.S.A.)

Potassium Chloride (KCL) (E. Merck, Darmstadt, W. Germany)

Penicillin (Dumex, Bangkok, Thailand)

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

Sodium bicarbonate (NaHCO_3) (BDH, England)

Sodium carbonate (Na_2CO_3) (J.T.Baker Chemical co.,
NY, U.S.A.)

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

Sodium hydroxide (NaOH) (E.Merck, Darmstadt, W.Germany)

Streptomycin (Thai meiji, Bangkok, Thailand)

Sulfuric acid (H_2SO_4) (E.Merck, Darmstat, W.Germany)

Trysin (E.Merck, Darmstadt, W.Germany)

Tween 20 (Sigma, MO, U.S.A.)

B. Antiserum

Fluorescein conjugated rabbit immunoglobulin to
human IgG (Dako, Glostrup, Denmark)

Goat anti mouse serum fluorescein conjugate (Cappel,
PA, U.S.A.)

Peroxidase conjugated rabbit immunoglobulin to human
IgG (Dako, Glostrup, Denmark)

C. Glasswares

Beaker (Pyrex, Corning, NY, U.S.A.)

Cylinder (Witeg, W.Germany)

Disposable 96 wells polystyrene microtiter plate
(flat bottom) certified plate (Nunc, Roskilde, Denmark)

Erlenmayer flask (Pyrex, Corning, NY, U.S.A.)

Glass tube (Pyrex, Corning, NY, U.S.A.)

Microcentrifuge tube (Treff AG, Schweiz, Switerland)

Polystyrene microtiter plate (Microwell module,
Nunc, Roskilde, Denmark)

Tissue culture bottle (Kotobuki, Tokyo, Japan)

Tissue culture multi-well plate (Flow, Connecticut,
U.S.A.)

D. Instruments

Analytical balance (Mettler PC 440, Zurich,
Switzerland)

Automatic pipet (EFLAB OY, Helsinki, Finland)

Centrifuge (IEC CENTRA-7R, Needham Hts., MA 02194,
U.S.A.)

ELISA reader, Titertek Multiscan (Flow, Helsinki,
U.S.A.)

Incubator (Memmert, W.Germany)

Mixer Vortex-Genie (Scientific Industries, NY, U.S.A.)

pH meter, PHM 83 (Radiometer, Copenhagen, Denmark)

Sonicator, Soniprep 150 (MSE, United, Kingdom)

Spectrophotometer, Coleman Junior II, model 6/35,
(IL, USA.)

Waterbath, Julabo TWB 12 (Seelbach, W.Germany)

APPENDIX II

REAGENTS AND PREPARATIONS

1. Reagents for cell culture.

1.1 Fetal bovine serum.

Fetal bovine serum was inactivated by heat at 56 °C (water bath) for 30 min and should be stored at - 20 °C.

1.2 HEPES buffer 1 M, pH 7.3

HEPES	235.3	gm
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Make up to 1000 ml with distilled water and adjust pH to 7.3 with 5 N NaOH.

The solution was sterilized by membrane filtration, and stored at 4 °C.

1.3 7.5% sodium bicarbonate.

NaHCO ₃	75	gm
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Deionized distilled water to	1000	ml
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The solution was sterilized by autoclave at 15 lb pressure for 15 min and stored at 4 °C.

1.4 Culture media.

1.4.1 Growth media (GM)

MEM 10x	10	ml
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Heat-inactivated FBS	10	ml
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Penicillin, 20,000 u/ml	0.5	ml
Streptomycin, 20,000 ug/ml	0.5	ml
Amphotericin B, 200 ug/ml	1.0	ml
7.5% NaHCO ₃	2.0	ml
HEPES, 1 M	2.0	ml
Deionized distilled water to 100		ml

The medium should be stored at 4 °C.

1.4.2 Maintenance medium (MM).

MEM 10x	10	ml
Heat-inactivated FBS	2	ml
Penicillin, 20,000 u/ml	0.5	ml
Streptomycin, 20,000 ug/ml	0.5	ml
Amphotericin B, 200 ug/ml	1.0	ml
7.5% NaHCO ₃	2.0	ml
HEPES, 1 M	2.0	ml
Deionized distilled water to 100		ml

The medium should be stored at 4 °C.

1.4.3 MEM without serum.

MEM 10x	10	ml
Penicillin, 20,000 u/ml	0.5	ml
Streptomycin, 20,000 ug/ml	0.5	ml
Amphotericin B, 200 ug/ml	1.0	ml
7.5% NaHCO ₃	2.0	ml
HEPES, 1 M	2.0	ml
Deionized distilled water to 100		ml

The medium should be stored at 4 °C.

1.5	Phosphate buffer saline, 0.15 M, pH 7.4		
	NaCl	8.0	gm
	KCl	0.2	gm
	Na ₂ HPO ₄ (anhydrous)	1.15	gm
	KH ₂ PO ₄	0.2	gm
	Deionized distilled water to	1000	ml

The solution was sterilized by autoclave at 15 lb pressure for 15 min and stored at 4 C.

1.6	Trypsin versene solution (TV-solution).		
	Trypsin	5.0	gm
	EDTA	2.0	gm
	NaCl	9.0	gm
	Deionized distilled water to	1000	ml

2. Reagent for indirect immunofluorescent antibody test.

2.1	Phosphate buffer saline, 0.15 M, pH 7.4		
	NaCl	8.0	gm
	KCl	2.0	gm
	Na ₂ HPO ₄ (anhydrous)	1.15	gm
	KH ₂ PO ₄	2.0	gm
	Distilled water to	1000	ml
	Adjust the pH to 7.4 and stored at 4 C.		

2.2	Counter stain.		
	Evans blue	0.01	gm
	Distilled water	300	ml
	The solution should be stored at 4 C.		

2.3 Buffered glycerol mounting medium.

Add 90 ml of glycerol to 10 ml of PBS, pH 7.4

3. Reagent for the detection of HSV antibody by ELISA test.

3.1 Coating buffer, pH 9.6

Na_2CO_3	1.59	gm
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NaHCO_3	2.93	gm
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NaN_3	0.2	gm
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Make up to 1000 ml with distilled water and adjust pH to 9.6 with 1 M NaOH.

Store at 4 C or room temperature for not more than 2 weeks.

3.2 Phosphate buffer saline-Tween(PBS-Tween), pH 7.4

NaCl	8.0	gm
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KH_2PO_4	0.2	gm
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Na_2HPO_4	1.15	gm
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KCl	0.2	gm
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Tween 20	1	ml
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Make up to 1000 ml with distilled water, adjust the pH to 7.4, and store at 4 C.

3.3 Phosphate buffer saline Tween-albumin (3%).

Dissolve 3 gm of bovine serum albumin (BSA) in 100 ml of PBS-Tween, pH 7.4

Prepare before use.

3.4 Substrate buffer for horseradish peroxidase
Phosphate-citrate buffer, pH 5.

Solution A : 0.1 M solution of citric acid

$\text{H}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$	21.0	gm
Distilled water	1000	ml

Solution B : 0.2 M solution of Na_2HPO_4

Na_2HPO_4	28.4	gm
Distilled water	1000	ml

Titrate solution A with solution B to pH 5
Store at 4 C.

3.5 Substrate solution.

Thirty-four mg amount of OPD is dissolved in 100 ml of phosphate-citrate buffer, pH 5; 40 μl of 30% H_2O_2 is added, and the substrate solution is used immediately. This substrate solution is light sensitive, and it is essential to shield it from strong light.

3.6 Reaction stopping solution, 4 N H_2SO_4 .

H_2SO_4 conc.	112	ml
Distilled water to	1000	ml



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