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APPENDIX

BUFFERS AND REAGENTS

BUFFERS:

Phosphate buffer saline (PBS), pH 7.4

Stock solution:

NaH_2PO_4 (0.2 M)	27.998	gm
Na_2HPO_4 (0.2 M)	35.598	gm

Add NaH_2PO_4 to Na_2HPO_4 dropwise to adjust the pH 7.4

Working solution:

Stocking solution	50	ml
NaCl	8.76	gm
Distilled water	1000	ml

0.2 M Cacodylate buffer, pH 7.4

$Na(CH_3)_2AsO_4.3H_2O$	42.8	gm
Distilled water	500	ml

Add 0.2 M HCl to adjust the pH 7.4 and make final volume 1000 ml with distilled water

0.1 M Cacodylate buffer, pH 7.4

0.2 M cacodylate buffer, pH 7.4	1	part
Distilled water	1	part

Veronal acetate buffer

Stocking solution:

NaCl	3.4	%
Sodium acetate	1.94	%
Sodium barbital	2.94	%

Working solution:

0.22 M veronal acetate buffer, pH 7.4

veronal acetate stock solution	5	ml
distilled water	13	ml
1 M $CaCl_2$	0.25	ml

add o.1 N HCl to adjust pH

0.11 M veronal acetate buffer, pH 7.4

0.22 M veronal acetate buffer	1	part
distilled water	1	part

Gold Buffer (for dilute Protein-A gold conjugate)

Tris-HCl	6.61	gm
Tris base	0.97	gm
NaCl	8.77	gm
Distilled water	800	ml

Adjust pH to 7.4 with 0.1 N HCl and bright volume to 1000 ml with distilled water.

REAGENTS:

Fixative:

1% Glutaraldehyde

50% glutaraldehyde (EM grade)	0.2	ml
0.2 M cacodylate buffer, pH 7.4	5	ml
Distilled water to make 10 ml		

2.5% glutaraldehyde

50% glutaraldehyde (EM grade)	0.5	ml
0.2 M cacodylate buffer, pH 7.4	5	ml
distilled water to make 10 ml		

2% Paraformaldehyde

Paraformaldehyde	2	gm
0.2 M phosphate buffer, pH 7.4	50	ml

Heat to 65°C with stirring, solution will be cloudy. Add a few drops of 1 N HCl to clear solution. Cool and add distilled water to make 100 ml final volume.

Periodate-Lysine-Paraformaldehyde (PLP)

Stocking solution:

1. 8% Paraformaldehyde

Paraformaldehyde	8	gm
Distilled water	100	ml

2. Lysine-phosphate buffer

To 0.2 M lysine-HCl in distilled water, 0.1 M dibasic sodium phospphate was added until the pH was 7.4

Working solution:

1 part of 8% paraformaldehyde and 3 parts of lysine-phosphate buffer were combined with 0.01 mole of sodium meta-periodate

Reduced Osmium

Stocking solution:

1. 4% Osmium tetroxide

Osmium tetroxide(OsO_4)	1	gm
Distilled water	25	ml

2. 2% $K_4Fe(CN)_6$

$K_4Fe(CN)_6$	0.2	gm
0.3 M cacodylate buffer,pH 7.4	10	ml
$CaCl_2$ (2.5 mM)	0.054	gm

Working solution:

Mix equal amount of 4% osmium tetroxide and 2% $K_2Fe(CN)_6$ (preparation before use)

Spurr resin (Embedding media)

Nonenyl succinic anhydride (NSA)	26	gm
Vinyl cyclohexane dioxide (ERL)	10	gm
DER resin	6	gm
Dimethylaminomethane (DMAE)	0.4	gm

0.5% Bovine serum albumin (for dilute 1°Ab)

Bovine serum albumin	0.5	gm
Phosphate buffer saline	100	ml

Dissolve and shake, store at 4°C

0.4% Trypan blue

Trypan blue	0.4	gm
NaCl	0.01	gm
K_2HPO_4	0.06	gm
Distlled water	100	ml

Disslove and shake, filter before use.

0.05% Trypsin

Stocking solution:

1. 0.02 % EDTA

NaCl	8	gm
KCl	0.2	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.2	gm
EDTA	0.2	gm
Phenol red	0.01	gm
Distilled water	1000	ml

Shank until all components are complete dissolved.

2. Phosphate buffer saline calcium magnesium free, pH 7.4 (PBS-CMF)

NaCl	8	gm
KCl	0.2	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.2	gm

Distilled water	1000	ml
Phenol red	0.01	gm

Shake until all component are complete dissolved. Adjust pH with 1 N NaOH.

3. 2.5% Trypsin

Trypsin	2.5	gm
PBS-CMF	100	ml

When trysin is dissolved in PBS-CMF, the color will change from red to yellow.

Working solution:

2.5% trypsin	2	ml
0.02% EDTA	50	ml
PBS-CMF	48	ml

Mix all solution into complete solution. For sterilization by filter with 0.22 μ milipore.

Staining solution (For EM thin section)

1. 0.5% Uranyl acetate

Uranyl acetate	0.05	gm
30% ethanol	10	ml

Dissolve and mix gentle, filter through 0.22 μm milipore. This solution is light sensitive. It is possible to be stored frozen in small aliquotes.

2. Lead citrate

Lead nitrate [$Pb(NO_3)_2$]	0.3325	gm
sodium citrate [$Na_3(C_6H_5)_7.2H_2O$	0.4400	gm
Distilled water	7.5	ml

Shake intermittently form 30 minutes, add 1 N NaOH dropwise 2 ml, shaking vigorously until the mixture is clear and add 2.5 ml of distilled water.

Toluidine blue stain (For EM thick section)

Sodium borate	2	gm
Distilled water	100	ml
Toluidine blue	1.5	gm

Shake to dissolve, filter before use.

Hematoxylin & Eosin (H&E) staining (For light microscopic examination)

Mayer's Hematoxylin

Hematoxylin crystal	1.0	gm
Distilled water	1000	ml
Sodium iodate	0.2	gm
Ammonium alum	50.0	gm
Citric acid	1.0	gm
Chloride hydrate	50.0	ml

Dissolve the alum in water,without heat, add the dissolved hematoxylin. Then add sodium iodate, citric acid and chloride hydrate. Shake until all components are dissolved completely. The final color of the stain is raddish-violet. This solution keeps well for months, filter before use.

Eosin-Phloxine solution

Eosin stocking

Eosin Y.(water soluble)	1	gm
Distilled water	100	ml

Phloxine stocking

Phloxine B.	1	gm
Distilled water	100	ml

Working solution

Eosin stocking	100	ml
Phloxine stocking	10	ml
95% ethanal	780	ml
Glacial acetic acid	4	ml

Culture media:

RPMI media

RPMI 1640 with L-glutamime, without $NaHCO_3$	10.41	gm
$NaHCO_3$	2.0	gm
Sterile water (for injection)	1000	gm
Penicillin-Streptomycin solution (penicillin 100μg/ml and streptomycin 100 IU/ml)	10	ml

Dissolve and shake until all component are complete dissolved. Forsterilization, filter through 0.22μm milipore.

RPMI hybrid media

RPMI 1640 with L-glutamine, without $NaHCO_3$	10.41	gm
$NaHCO_3$	2.0	gm
Sterile water (for injection)	1000	ml
Penicillin-Streptomycin solution	10	ml
L-glutamine	0.1	gm
D-glucose	2.0	gm
Pyruvic acid	0.11	gm

Dissolve and shake until all component are complete dissolved. For sterilization, filter through 0.22 μm milipore .

AUTHOR BIBLIOGRAPHY

Miss Warunee Dansithong was born on December 31st, 1964 in Phitsanuloke Province. Since she graduated with the Bachelor degree of Science at Faculty of Associated Medical Science, Chiang Mai University in 1987, she was enrolled as Medical Technologist at deparment of internal medicine, Faculty of Medicine, Prince of Songkhla University. She entered the graduate programme for Master degree of Biotechnology at Chulalongkorn University in 1992. During her study, she was a candidate for U.D.C. Scholarship.