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APPENDIX

APPENDIX

Buffers and Reagents

1. RPMI 1640 stock solution	1	liter
RPMI powder	10.4	g
$NaHCO_3$	2	g
ddH_2O	900	ml

Adjust pH to 7.2 with 1 M HCl

Add ddH_2O to 1 liter and sterilize by filtering through a 0.45 μm membrane filter.

2. HBSS stock solution	1	liter
HBSS powder	9.52	g
$NaHCO_3$	0.35	g
ddH_2O	900	ml

Adjust pH to 7.2 with 1 M HCl

Add ddH_2O to 1 liter and sterilize by filtering through a 0.45 μm membrane filter.

3. 10X Phosphate Buffered Saline (PBS)	1	liter
NaCl	80	g
KCl	2	g
$NaHPO_4$	9.136	g
KH_2PO_4	2	g
ddH_2O	900	ml

Adjust pH to 7.4 with 1 M HCl

Add ddH_2O to 1 liter and sterilize by autoclave.

4. 1X Phosphate Buffered Saline (PBS)	1	liter
10XPBS	100	ml
ddH_2O	900	ml

Sterilize by autoclave.

5. 10 % Buffered Formalin Acetate solution	1	liter
Paraformaldehyde powder	10	g
1 M NaOH	1	ml
Formaldehyde	40	ml
10X PBS	100	ml
warm ddH_2O	800	ml

Adjust pH to 7.00 with 1 M HCl

Add ddH_2O to 1 liter and sterilize by filtering through a 0.45 μm membrane filter.

6. PBS / 1 % Paraformaldehyde fixative 1 liter

1 X PBS	600	ml
10 % Buffered Formalin Acetate	400	ml

Adjust pH to 7.1 and sterilize by filtering through a 0.45 μm membrane filter.

7. 200 mM L-Glutamine

L-Glutamine	29.22	g
ddH_2O	1,000	ml

Sterilize by filtering through a 0.45 μm membrane filter.

Aliquot and store in polypropylene centrifugue tube at – 20° C

** Do not heat; Since heat will destroy glutamine.

8. Hoeschts 33258 Dye stock solution

Hoeschts dye	0.005	g
ddH_2O	50	ml

Store in polypropylene centrifuge tube at – 20° C.

9. Hoeschts Dye solution (for 8 slides)

Hoeschts Dye 33258 stock solution	180 µl
1 X PBS	45 ml

** Prepare freshly before use.

10. 2 µl / ml Heparin in HBSS

HBSS stock	22.5 ml
Heparin	45 µl

11. Complete RPMI 1640 medium 69 ml

RPMI stock	65 ml
L-Glutamine	338 µl
Gentamycin	16.25 µl
Fetal Bovine Serum	3.58 ml

12. HBSS / 2 µl / ml Heparin / 1 % Fetal Bovine Serum

HBSS	49.5 ml
Heparin (5,000 i.u./u.i./ml)	100 µl
Fetal Bovine Serum	0.5 ml

13. Stop Wash Buffer (Apoptag kit)	400	ml
Stop Wash Buffer	11.43	ml
ddH_2O	388.57	ml
Store at 4°C		

14. TdT mixture (for 8 slides)		
Reaction Buffer	152	µl
TdT enzyme	64	µl
** Prepare freshly before use.		

15. FITC mixture (for 8 slides)		
Blocking solution	112	µl
Anti-Digoxygenin	98.67	µl
** Prepare freshly before use.		

16. Hoeschts Dye solution		
Hoeschts Dye solution	180	µl
1 X PBS	45	ml
** Prepare freshly before use.		

17. 1 M NaOH	100	ml
NaOH	4	g
ddH_2O	100	ml

Store at room temperature.

BIOGRAPHY

Miss Janpen Udom was born on June 6, 1974 in Phangnga province, Thailand. She received the degree of Diploma in Nursing Science Equivalent to Bachelor of Science in Nursing, 1996 from Ministry of Boromarajjionani College of Nursing, Trang, Thailand. She has enrolled at Chulalongkorn University in graduate program for the Degree of Master of Science in Physiology and graduated in 2002.