

VIII. APPENDIX

1. Ficoll-Hypaque solution

A. 9% Ficoll solution

Ficoll powder	90.0 g.
Distilled water	1,000.0 ml

Mixed the solution using magnetic stirrer on hot plate until it has completely dissolved. Allow to cool at room temperature.

B. 34% Hypaque solution

Hypaque (50%)	30 parts
Distilled water	14 parts

Mixed well and stored at 4 °C.

C. Ficoll - Hypaque solution

9% Ficoll	24 parts
34% Hypaque	10 parts

Mixed well and sterilized by passage through the 0.2 μ millipore filter and stored at 4 °C.

2. Phosphate Buffer Saline (PBS) pH 7.4

NaCl	7.65	g.
Na ₂ HPO ₄	1.16	g.
NaH ₂ PO ₄ . 2H ₂ O	0.315	g.
Distilled water	1,000.0	ml.

Mixed completely until salts are dissolved. The pH should be 7.2 ± 0.05 . pH can be adjusted by 0.1 N HCl or 0.1 N NaOH. Sterilized by passage through 0.2 μ millipore filter and stored at 4 °C.

3. RPMI-1640 tissue culture medium

RPMI-1640 powder	10.4	g.
NaHCO ₃	2.0	g.
Distilled water	1,000.0	ml.
gentamicin 40 mg %	1.0	ml.

Mixed completely and adjust the pH 7.2-7.4 with 1 N HCl. Sterilized by millipore filtration and stored at 4 °C.

4. Preparation of complete RPMI-1640 medium

Heat inactivated (56 ° C, 30 min) fetal bovine serum 10 ml was added to 90 ml of RPMI - 1640 medium which supplemented with 12.5 mM HEPES buffer, 5×10^{-5} M 2-mercaptoethanol (2-ME), 1 mM sodium pyruvate. Mixed thoroughly and ready to be used or stored at 4 °C.

5. Preparation of tritiated-thymidine (8 μ Ci/ml)

40 μ l of [3 H]-thymidine (stock 47 Ci/mmol; Amersham, batch 304) was diluted in 5 ml RPMI-1640 to a final concentration of 8 μ Ci/ml Mixed thoroughly and ready to be used or stored at 4 $^{\circ}$ C.

6. Preparation of scintillant

PPO (2,5-diphenyloxazole, Sigma)	10.0 g.
POPOP (1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene, Sigma)	0.25 g.
Toluene (Merck)	2.5 L.

Mixed well until the PPO and dimethyl POPOP crystals were completely dissolved and the scintillant was ready to use. Stored at room temperature in dark container.

7. Brain heart infusion (BHI) agar

BHI agar (dehydrated)	52.0 g.
Distilled water	1,000.0 ml.

Melt, disperse in tube and autoclave at 121 $^{\circ}$ C, 15 lbs for 15 min. Allow tubes cool in slant position.

8. BHI broth

BHI	37.0 g.
Distilled water	1,000.0 ml.

Melt, disperse 50 ml in each 250 ml-Erlenmeyer flask.

Autoclave at 121 $^{\circ}$ C, 15 lbs for 15 min.

IX. CURRICULUM VITAE

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