

APPENDIX

1. Preparation of 0.01 M phosphate buffer saline pH7.2

$\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$	2.9266	gm
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	0.3250	gm
NaCl	7.65	gm
Distilled water	1,000	ml

Mix thoroughly until salts are dissolved. Adjust pH of the solution, if needed, with 1 N HCl or 1 N NaOH. The solution was sterilized by autoclaved and stored at 4°C.

2. Preparation of Ficoll-Hypaque solution

9% Ficoll solution:

9 gm of Ficoll powder (Ficoll-400, Pharmacia, Sweden) were dissolved in 100 ml distilled water.

34% Hypaque solution:

30 ml of 50% aqueous solution of diatrizoate sodium injection (Hypaque[®] sodium 50%, Winthrop, USA) were diluted with distilled water to get 34% Hypaque solution.

Ficoll-Hypaque solution:

24 parts of 9% Ficoll solution were mixed with 10 parts of 34% Hypaque solution. This mixture were adjusted to get the density of 1.075-1.077 then the solution was sterilized by millipore filtration and stored at 4°C.

3. Preparation of RPMI 1640 medium

RPMI 1640 powder (with L-glutamine, without sodium bicarbonate and antibiotic, Flow Laboratories, Mc Lan, Va) 10.4 gm were dissolved in 900 ml of distilled water, then 2.0 gm of sodium bicarbonate and one milliliter of 100,000 units of penicillin G sodium and 100,000 ugm of streptomycin sulphate solution were added. The solution was adjusted to 1,000 milliliters with distilled water, sterilized by millipore filtration and stored at 4°C.

4. Preparation of complete RPMI 1640 (CRPMI) medium

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

5. Preparation of Alsever's solution

Glucose	10.25	gm
Sodium chloride	2.1	gm
Sodium citrate dehydrate	4.0	gm
Distilled water	500.0	ml

Mix thoroughly until salts are dissolved and adjust pH to 6.1 with 1 M citric acid or 1 M NaOH. This solution was sterilized by millipore filtration and stored at 4°C.

6. Preparation of hypotonic ammonium chloride solution

NH ₄ Cl	8.29	gm
KHCO ₃	1.00	gm
EDTA	0.0372	gm
Distilled water	1,000	ml.

Mix thoroughly until salts are dissolved and adjust pH to 7.2 with 1 M HCl or 1 M NaOH. This solution was sterilized by millipore filtration and stored at 4°C.

7. Preparation of Ficoll-Hypaque solution for removing dead cells

14% Ficoll Solution:

14 gm of Ficoll powder (Ficoll-400, Pharmacia, Sweden) were dissolved in 100 ml distilled water

32.8% Hypaque solution:

30 ml of 50% aqueous solution of diatrizoate sodium injection (Hypaque[®] sodium 50%, Winthrop, USA) were diluted to get 32.8% Hypaque solution with distilled water.

Ficoll-Hypaque solution:

12 parts of 14% Ficoll solution were mixed with 5 parts of 32.8% Hypaque solution and were adjusted to get the density of 1.09. The solution was sterilized by millipore filtration and stored at 4°C.

8. Preparation of 0.2% nigrosin solution

Nigrosin powder	0.2	gm
0.01 M PBS, pH 7.2	100.0	ml

Mix thoroughly and non - sterilely filtration. This solution was stored at room temperature.

9. Preparation of scintillant

PPO (2, 5-diphenyloxazole, Sigma)	10	gm
dimethyl POPOP {1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benylene, Sigma}	0.25	gm
Toluene	2.5	let.

Mix 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.

BIOGRAPHY

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