

APPENDIX: PREPARATION OF HUMAN ERYTHROCYTES

1. Prepare the Puck's Saline Glucose (PSG) medium (see recipe below), mix well, adjust pH to 7.2, and adjust the volume to 1 L with distilled, deionized water. Filter sterilize using a 0.22 µm filter and store at 4°C.

2. Prepare the PSG+G solution following the recipe below and mix well.

Puck's Saline Glucose Medium

CaCl ₂ • 7H ₂ O	0.016 g
KCl	0.40 g
KH ₂ PO ₄	0.15 g
MgSO ₄ • 7H ₂ O	0.15 g
NaCl	8.0 g
Na ₂ HPO ₄ • 7H ₂ O	0.29 g
D-glucose	1.10 g
Phenol red	0.005 g
Distilled, deionized water to	1 L

PSG+G Solution

Puck's Saline Glucose Medium	500 mL
D-glucose	10 g
Antibiotic/Antimycotic Solution (ATCC® PCS-999-002™)	5 mL

3. Filter sterilize using a 0.22 µm filter and store at 4°C.
4. Aseptically, wash donor blood three times by centrifugation at 600 to 800 × g for 15 minutes at 4°C in RPMI 1640 medium.
5. After each wash, aseptically remove the supernatant, consisting of the plasma and buffy (leukocyte) layers located on the top of the red blood cell (erythrocyte) pellet.
6. After the last wash, aseptically resuspend human erythrocytes in sterile PSG+G solution at a concentration of 50% erythrocytes. The human erythrocytes in PSG+G solution may be stored at 4°C until use, for a maximum of two weeks.