RBC Lysing Solutions and Cell Lysing Procedure

Ammonium chloride lyse (10X concentration)

NH4Cl (ammonium chloride)  8.02gm
NaHCO3 (sodium bicarbonate)  0.84gm
EDTA (disodium)  0.37gm

QS to 100ml with Millipore water. Store at 4°C for six months.

Working solution
Dilute 10ml 10X concentrate with 90 ml Millipore water. Refrigerate until use.

Procedure
1. Obtain a whole blood specimen in a heparin tube.
2. Aliquot 1ml blood into 15ml conical centrifuge tube.
3. Fill tube to capacity with fresh cold lysing solution.
4. Invert or rock for ~10 minutes at room temperature until liquid is clear red.
5. Spin at 4°C for 10 minutes at 250 x g.
6. Decant supernatant and allow tubes to drain briefly.
7. Resuspend cell by raking gently across a tube rack.
8. Wash cells and combine multiple tubes with 10ml cold PBS/2% FCS.
9. Spin, decant, and resuspend as above (steps 5-7).
10. Count cells and adjust cell concentration to ~2-4x 10^6/ml.