General - RBC Lysis Guidelines

Scope:

This procedure applies to generating white blood cell (WBC) isolates from whole blood by removing RBC interference through preferential RBC lysis.

Overview:

This guide outlines a general RBC lysis protocol based on St Michaels' PBMC "Preparation for Flow Cytometry" document. In our experience, RBC lysis buffers vary considerably in their efficacy at removing RBC 's. This is purportedly due to variations in originating sample (e.g. Sodium Citrate affect on lyse buffer osmolarity). Starting with a buffy coat isolation dramatically reduces the starting RBC concentration, thereby increasing the efficacy of the RBC Removal.

Buffy Coat Isolation:

- Obtain a Fresh Whole Blood sample
- Spin the whole blood at 200xg for 10 minutes with the BRAKE OFF
- Remove the leukocyte band (the buffy coat). This is the interface layer between the RBC's (bottom) and the plasma (top).
- Aliquot leukocyte band into a 15ml centrifuge tube

RBC Lysis Protocol:

Ammonium Chloride Lysis Buffer Recipe:

Note: a 10X buffer can be made and diluted to 1x immediately before use. The recipe below is for a 1x buffer

- NH_4Cl - 8.02g
- $NaHCO_3$ – 0.84g
- EDTA (disodium) – 0.37g
- dissolve in 100ml double distilled H_2O (ddH_2O)
- pH adjust to 7.2
- Filter (.45mm) and store at 4°C for up to 6 months

Protocol:

1. Pre-warm lysis buffer to 37°C
2. Fill 15ml leukocyte tube to capacity with lysing solution.
3. Invert or rock for ~10min at room temperature until liquid is clear red.
4. Centrifuge at 4°C at 250xg for 10 minutes.
5. Carefully remove supernatant with aspiration pipette.
6. Add 5ml cold PBS/2% FBS.
7. GENTLY re-suspend cells with a pipette.
8. Centrifuge at 4°C at 250xg for 10 minutes.
9. Count cells and adjust concentration to the desired level for staining (typically 1e6 cells/ml)