Moxi GO II – RBC Lysis – Cell Surface Immunolabeling

Scope:

This procedure applies to the antibody (e.g. anti-CD marker) labeling of white blood cells (WBC's) isolated from a peripheral whole blood sample or other RBC contaminated sample by 1.) (Optional, whole blood only) Performing a buffy coat isolation., 2.) Applying an antibody label, and 3.) Lysing the residual red blood cells (RBC's).

Overview:

As RBC's outnumber the WBC's in a whole blood sample by ~1000x, it is necessary to eliminate the RBC interference prior to running on the Orflo Flow Cytometers. In our experience, RBC lysis buffers vary considerably in their efficacy at removing RBC 's. While the BioLegend RBC Lyse Buffer (used in this protocol) is better than most, we have also found that starting with a buffy coat isolation dramatically reduces the starting RBC concentration, improving the overall results.

Reagents:

- Purified Water (i.e. Double-Distilled)
- 10x RBC Lyse Buffer (Biolegend cat#420301)
- Cell Staining Buffer (BioLegend cat # 420201)
- Compatible fluorophore conjugated antibody
  - ***525/45nm Filter (PMT1)*** – FITC or Alexa Fluor 488 labeled antibody
  - ***561nm/LP Filter (PMT2)*** - Phycoerythrin (PE/R-PE) labeled antibody (e.g. PE anti-human CD4 - BioLegend cat # 300507) – *Note: Substitute Antibodies that are compatible with 532nm excitation and 561nm/LP emission can also be used instead of PE.*
- Moxi Cyte Flow Reagent (Orflo MXA079/MXA080)

Pre-Prep:

- Warm water and RBC Lyse Buffer to room temperature
- Dilute RBC lyse buffer to 1x, E.g.
  - Add 9ml of purified water to a 15mL centrifuge tube
  - Add 1ml of 10x RBC Lyse Buffer to the above tube

Note: The water purity is very important in creating the lysis buffer as the efficacy can dramatically change with pH and osmolarity.

1. Buffy Coat Isolation (optional, whole blood only):

1.1. Spin the whole blood at 1500 x g for 10 minutes with the BRAKE OFF

1.2. Remove and discard the majority (all but 2-3mm) of the top (amber/translucent) plasma layer, taking care not to disturb the buffy coat layer (at the interface of the red, RBC layer).

1.3. Use a large pipette (cut tip to widen if necessary) to extract the leukocyte band (the buffy coat). Notes:

- The buffy coat is the (cloudy/white) interface layer between the RBC's (red) and the plasma (amber/translucent).
- To ensure minimal loss of WBC's, it is necessary to include the residual plasma as well as a small portion of the RBC layer in the extracted buffy coat sample.

1.4. Aliquot buffy coat into a 15ml centrifuge tube
1.5. Dilute cells to a concentration of ~5e6 cells/ml. (e.g. for 3ml starting volume of whole blood, add ~2ml of staining buffer to extracted buffy coat sample)

2. Antibody Label:

2.1. Quick spin (e.g. 500 x g, 15 seconds) antibody vials for maximum volume.
2.2. Prepare 15mL centrifuge tubes with the antibody labels. *Note: Prepare as many samples as would be required for proper compensation (e.g. 4 samples: Dual-label, antibody 1 positive control, antibody 2 positive control, negative control (e.g. isotype control or unlabeled)*
2.3. Aliquot 100 µl of the buffy coat sample or whole blood to each prepared 15ml tube. Pipette up and down to mix sample and antibody well.
2.4. Incubate for 15-20 min at 4°C, protect from light.

3. RBC Lyse:

3.1. Add 2ml of the room temperature, 1x RBC lysis buffer to each tube. Note: Immediately after adding it to the sample, vortex the sample (e.g. level 3) for 1-2 seconds
3.2. Incubate at 37°C in the dark for 15 minutes.
3.3. Add 3ml Staining Buffer and invert 2x.
3.4. Spin the sample at 350 x g for 5 minutes with the BRAKE OFF.
3.5. Carefully remove the supernatant.
3.6. Add 5ml staining buffer and pipette to re-suspend cell pellet (Note: If using a 1mL Pipette, add just 1ml staining buffer, pipette the sample, then add 4ml additional staining buffer with a fresh tip)
3.7. Spin the sample at 350 x g for 5 minutes with the BRAKE OFF.
3.8. Carefully remove the supernatant.
3.9. Re-suspend sample to an expected ~1e5 – 5e5 cells/ml by adding ~1-2ml of Staining Buffer.
3.10. *(Optional) 20µl of Moxi Cyte Flow Reagent*.
3.11. Invert 10x to mix and analyze with the Moxi GO II using the "Open Flow Cytometry" or "GOFlow" Assays.