Moxi Flow - Cell Surface – Immunolabeling Protocol

Reagents:

- Cell Staining Buffer (BioLegend cat # 420201)
- Labeled Antibodies:
  - Primary label:
    - Phycoerythrin (PE) labeled primary antibody (e.g. PE anti-human CD4 - BioLegend cat # 300507))
    -
  - Secondary label:
    - Purified antibody (e.g. Purified Mouse IgG1, anti-human CD4 - BioLegend cat # 344601)
    - Phycoerythrin (PE) labeled secondary antibody (e.g., PE goat anti-mouse IgG - BioLegend cat # 405307)
- Moxi Cyte Flow Reagent (Orflo MXA079/MXA080)
- (Optional) Human TruStain FcX - Fc Receptor Blocking Solution (BioLegend cat # 422301)
- *(OPTIONAL)* Accutase (Orflo MXA020) or Accumax (Orflo MXA021) dissociation reagents2

Cell Isolation/Preparation

Prepare cells into a single-cell suspension free of large (>30um) particulate.
Notes:

- Primary harvests (e.g. blood or tissue extractions):
  - If large extracellular debris is present, pass sample through a 40um cell strainer or FLOWMI strainer prior to processing to remove it.
  - If large amounts of RBCs are present in the sample, an appropriate RBC lyse method must be applied to get proper counts (RBCs will mask the signal of the cell population of interest due to their extremely high concentration levels).
- Cell aggregation/Clustering – Clusters and aggregates can be dissociated through a combination of:
  - Protease treatment – suspend cells in an appropriate dissociation reagent (e.g. Accumax or Accutase) for a minimum of 5 minutes.
  - Pipette trituration (repeatedly, e.g. 10x, pull a substantial fraction of the cell sample in and out of a pipette tip). If a dissociation treatment was used, perform this step while cells are still suspended in the dissociation reagent.

Primary Antibody Staining Protocol

1. Suspend cells at density of 2-5 x 10^6 cells/ml in cell staining. (Note: For PBMC Samples, this would be following the last wash of the PBMC's, re-suspend the pellet directly into the staining buffer). Combine pellets if necessary. Verify counts using the Moxi Z or the Moxi Flow instruments.
2. Aliquot 100 µl of cells (2-5 x 10^5 total cells) into a 1.5ml microfuge tube.

(Optional) Block Fc-Receptors *(recommended for reducing nonspecific staining of antibodies)*

- i. *Add 1 test volume (typically 5 µl) of Fc Receptor Blocking Solution to 100 µl of cells.*
- ii. *Incubate for 10 minutes at room temperature.*

3. Quick spin (e.g. 500 x g, 30seconds) antibody vials for maximum volume.
4. Add 1 test volume (typically 5 µl) of primary antibody label to 100 µl cells.
5. Vortex gently.
6. Incubate for 15-20 min at 4°C, protect from light.
7. Wash 2X with 2ml of Cell Staining Buffer by centrifugation at 350 x *g* for 5 minutes at ≤18°C.
8. FOR DIRECTLY CONJUGATED ANTIBODIES:
   Re-suspend pellet in 1ml of cell staining buffer and add 20µl of Moxi Cyte Flow Reagent. Invert 10x to mix. Analyze with Moxi Flow using the "*Open Flow Cytometry*" app.

   FOR PURIFIED ANTIBODIES:
   Re-suspend pellet in residual buffer (typically 40-50µl) and add Cell Staining Buffer to 100µl total volume. Proceed to Secondary Antibody Staining.

<u>Secondary Antibody Staining</u>

9. Add 1 test volume (typically 2-5 µl) of 2° Ab (conc. 0.2µg/ml) to 100 µl cells (≤0.5µg per million cells in 100 µl).
10. Incubate for 15-20 minutes at 4°C, protect from light.
11. Wash 2x with 2ml of Cell Staining Buffer.
12. Re-suspend pellet in 1 ml of Cell Staining Buffer.
13. Add 20µl of Moxi Cyte Flow Reagent. Invert 10x to mix.
14. Analyze with Moxi Flow using the "*Open Flow Cytometry*" app.