Direct staining flow cytometry protocol for primary human isolates (PBMCs or Whole Blood)

1. Harvest, wash the cells and adjust cell suspension to a concentration of 1 x 10^6 cells/mL in ice cold PBS, 10% FCS, 1% sodium azide. Cells are usually stained in polystyrene round bottom 12 x 75 mm^2 Falcon tubes. However, they can be stained in any container for which you have an appropriate centrifuge e.g. test tubes, eppendorf tubes, and 96-well round bottomed microtiter plates. In general, cells should be centrifuged sufficiently so the supernatant fluid can be removed with little loss of cells, but not so hard that the cells are difficult to resuspend. **If you are using whole blood, AMREPFlow strongly recommend using a Red Blood Cell (RBC) Lysis Buffer (either hypotonic, ammonium chloride or any commercial lysis buffers will suffice). RBCs can and will overshadow (or swamp) your population of Leukocytes, making them hard to clearly indentify**.

We recommend staining with ice cold reagents/solutions and at 4°C, as low temperature and presence of sodium azide prevent the modulation and internalization of surface antigens. Internalization can cause a loss of fluorescence intensity.

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2. Add 0.1-10 µg/mL of conjugated primary antibody. Dilutions, if necessary, should be made in 3% BSA/PBS (Propidium iodide can also be added at this point for dead cell exclusion). The amount of antibody that needs to be added should be determined by an antibody titration step. (https://www.amrepflow.org.au/education/antibody-titration)
3. Incubate for at least 30 min in dark at room temperature or 4°C. This step will require optimization.
4. Wash the cells 3 x by centrifugation at 400 g for 5 min and resuspend them in 500 µL to 1 mL of fixative (such as 0.5-1% paraformaldehyde for 15-30mins. For known infectious samples, use 3-4% paraformaldehyde for 2-3 hours)
5. Always wash out the fixative post usage as this can affect fluorophore stability and fluorescence intensity.

   For general immunophenotyping purposes if you're using AMREPFlow instruments you MUST fix your sample.

   If you require to perform functional studies on live human samples, you must provide a Risk Assessment Document which will be reviewed by the Flow OHS Committee before work can commence.