Guidelines for the Hands-on Training on FCRC Analyzers

1. **Ice bucket** with samples covered with aluminum foil

   Note: *All the samples (unless otherwise specified by the protocol) should remain at +4 °C protected from light*

2. Plenty of the **unstained cells**. Cell concentration to be 5 x 10^6/ml. 1 ml volume should be enough. We will use them to establish the Application Settings. Useful Tips to Improve Sample Quality could be found at **Sample Preparation**

   Note: *These* unstained *cells should fully match the sample origin and preparation to be used in the future data acquisition on the FCRC analyzers or cell sorting*

3. **Cell samples stained with different concentrations of antibody** in order to find the proper titer for each antibody:

   a. For the antibody previously not used in the lab – bring "wide" titration
      For example: 3x; x; 1/3x; 1/9x; 1/27x; 1/81x (where "x" is the concentration suggested by the vendor)
   b. For the antibody previously used in the lab – bring "narrow" titrations
      For example: 3y; y; 1/3y; 1/9y (where "y" is the concentration suggested by the Labmates)
   c. For Live Dead Fixable Dyes - bring "narrow" titration
      For example: 3z; z; 1/3z (where "z" is the concentration suggested by the vendor). Make sure to follow the vendor's staining protocol (time, temperature, special buffer)
      Note: *Cell concentrations to be 5 x 10^6/ml. 1 x 10^6/ml in 200 ul volume should be enough*

4. 15 ml tube with the **"Staining Buffer"** (**Buffer Suggestions**) to use in case we need to dilute the samples or titer non-fixable Dead Cell Exclusion Dye (DCE)

5. 1.5 ml of the 1 ug/ml **stock of the non-fixable DCE** (for example, DAPI, 7AAD, PI, To-Pro3, etc.) to prepare titration and stain samples

   Note: *These dyes remain in the "Staining Buffer" with single cell suspension (no wash step)*

6. **Automatic pipets** (20, 200 and 1000 ul)

   Note: *FCRC doesn't lend pipets in the Analysis room, but offers tips*

7. **Sharpie marker** (to label tubes)