Guidelines for the Hands-on Training on BD Canto II

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 should 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 link sample preparation
   Note: *These unstained cells should fully match the sample origin and preparation to be used in the future data acquisition on the BD Canto II, LSR Fortessa or for cell sorting.*

3. Cell samples stained with different concentrations of antibody in order to find the proper amount 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: 2z; z; 1/2z (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 µl volume should be enough.*

4. 15 ml tube with the “FACS Buffer” link buffers 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 µg/ml stock of the non-fixable DCE (for example, DAPI, 7AAD, PI, ToPro3, etc.) to prepare titration and stain samples.
   Note: *These dyes remain in the “FACS Buffer” with single cell suspension (no wash step).*

6. Automatic pipets (20, 200 and 1000 µl) and tips.
   Note: *FCF-Berg doesn't lend pipets and tips in the Analysis room.*

7. Sharpie marker (to label tubes).