User Guidelines for Analyzers

Analyzers

The FCF Berg provides 2 analyzers, the BD Canto II and BD LSR Fortessa. The Canto II is able to measure 8 colors at once. The LSR Fortessa can measure up to 15 colors. Unless your experiment requires the measurement of more than 8 colors at once, it is recommended to use the Canto II.

Booking regulations for Canto II

In order to book an appointment, an account in the PPMS booking portal is mandatory (please contact the FCF staff). Before the first use of the analyzer, you have to fill out the "Analyzer User Sheet" and absolve the basic training at the FCF-Berg, which will take about 2-3 weeks. Therefore, please send an E-mail to the FCF-Berg.

Booking regulations for LSR Fortessa

To use the LSR Fortessa, you first have to proof that you are able to handle the Canto II. Then the FCF-Berg staff will activate your PPMS-account for booking the LSR Fortessa and will give an introduction upon request.

Sample preparation

Please avoid any mechanical detachment of the tissue/adherent cells. If possible, use an enzymatic detachment method or incubate in Calcium/Magnesium-free media. It is essential that you have a single cell suspension, to avoid clogging of the flow cell. Therefore, the cells should be filtered with cell strainers (35 or 40 µm) immediately before measurement. It is recommended to do a dead/live staining. The cells need to be measured in 12x75 mm standard FACS tubes.

Filtering

Falcon® 12x75mm, 5 ml polystyrene tube with cell strainer cap (35 µm), sterile, 25/pack, 500/case
Product #352235

Analyzing buffer

Some basic recipes for analyzing buffers are given below. However, they may need further optimization (e.g. concentrations) dependent on the cell type.

- 1x PBS (Ca^{2+}/Mg^{2+} free), 0.5 - 2 mM EDTA, 1-2% FCS, 0.2 µm filtered, store at 4 °C
- 1x PBS (Ca^{2+}/Mg^{2+} free), 1 mM EDTA, 25 mM HEPES pH 7.0, 1% FCS (heat-inactivated) or BSA, 0.2 µm filtered, store at 4 °C

Controls

It is recommended to use the following controls:

- *Negative control*

Please always bring along cells that do not express your antigen, e.g. non-transfected or better mock-transfected cells. Concerning surface stainings, always bring along unstained cells otherwise you will not be able to set the cutoff appropriately.

- *Compensation controls for multicolor setup*

Use single stainings for every antibody if you are using fluorophores with overlapping emission spectra

- If **cells** are used as single color controls
  - Unstained cells
  - Titration of the Dead Cell Exclusion dye on the "partially killed" cells
  - All separate single color stained cell controls

- If **beads** are used as single color controls
  - Unstained Cells
  - Unstained beads
  - All separate single color stained bead controls

- *Gating controls*

Fluorescent Minus One (FMO) controls, as proper way to evaluate the background in certain channel and set sorting gates appropriately