July 29, 2024

Issue 13

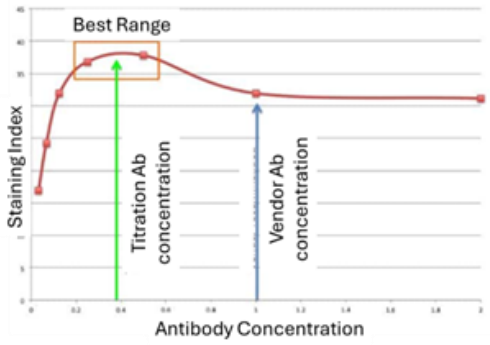
Research Flow Cytometry Facility
Quarterly Newsletter

Information Provided by your CCHMC RFCF

Increase Reproducibility & Reliable Results

Titrating antibodies optimizes the flow cytometry process to yield the best possible results. Antibody titration will reduce background noise, minimize nonspecific binding, enhance resolution of populations, increase reproducibility, decreases cost, and increase experimental efficiency. The best antibody concentration yielding the optimal positive population signal along with the lowest negative signal is determined by antibody titration. Excessive antibody increases background signal while too little antibody results in a decrease in positive signal; either will reduce the sensitivity of the fluorescent measurements and can alter experimental results. Antibodies are sold with a recommended dilution which is a great starting point; however, that concentration may not be optimal for your cell type or protocol.

Titration Curve:
Optimal concentration identified is ½ of vendor's recommendation



Many sources are available for titration experiment protocols. Antibody titration experiments should match the conditions used in your investigation. This includes the same cell type, a single incubation time, staining procedure, fixation, total volume and number of cells. A viability dye can be added and FC block should be used to prevent FC-mediated binding. The cells are then stained in a series of antibody dilutions. A suggestion is to start with twice the vendor recommended concentration (ug/mL) and do 8 serial 2-fold dilutions. A suggested gating strategy includes a time plot, scatter plot, doublet discrimination plots, and a live cell plot followed by a plot gating the fluorescent positive and negative cells. Record at least 1,000 live cells expressing your marker of interest and collect unstained cells as well. The SI (stain index or the separation index) is calculated using the median fluorescent intensity statistical data to determine optimal antibody concentration. The dilution providing the brightest staining with least background (highest SI) should be chosen. Titrations should be done for each antibody and when a new antibody lot of antibody is purchased.

Antibody Titration Protocol from Current Protocols in flow cytometry.
Flow TechNotes Titrating Antibodies for Flow Cytometry.
Flow_TechNotes_FlowJo Antibody Titrations_20170918
Cytek Antibody Titration Protocol or Video

Turning off the A5 SE

The A5SE lasers must be turned off between users. The more powerful lasers burn a hole in the flow cell when users fail to turn the instrument off.
When you come to use the A5SE, you need to turn the lasers on using the Coherent software. Do this while you are cleaning the instrument, which will give the lasers time to warm up. **Once you are done, even if someone is after you, please go back into the Coherent software and turn the lasers off**, then log out of the computer. If there is no one behind you, please turn the instrument off.

Panel Design & FluoroFinder

A good fluorescent panel using fluors with little spreading into each other is essential for reliable reproducible fluorescent data. Online panel builders can assist in creating fluorescent panels. One of these is FluoroFinder. FluoroFinder helps design and optimize fluorescent experiments for flow cytometry, immunohistochemistry, ELISAs, microscopy, and western blots. FluoroFinder collaborates with the scientific community to provide a platform to assist in fluorescent experiment design. The program has the largest database of commercially available antibody products and fluorophore data from many manufacturers. You can register individually for a basic plan or use the **CCHMC site license** purchased by the RFCF by emailing Celine Silva Lages. The program includes a spectra viewer, panel builder tools customized to CCHMC instrument configurations, AI feature, analysis of panel, RFCF access to your panels for assistance, inventory management, trainings and workshops.

Help Us Help You! Please complete a 3-question survey to tell us how you would like to receive panel design assistance from the RFCF.

GLIIFCA / ORVCA Annual Conference

Sept. 12-15, Covington, KY. Cincinnati Marriott at RiverCenter:
GLIIFCA / ORVCA Annual Conference
Abstract submission is now open until August 2nd.

Revoked Access

If you are unable to schedule RFCF instruments on Stratocore, your rights have been revoked. Please take the Laser Safety - Research Flow Cytometry Facility (TS_08115) course. Email Celine Silva Lages or RFCF staff the completion confirmation to get your rights reinstated.