Flow Post-its
Kathy Daniels

Antibody Validation

June 2020

Flow Cytometry commonly utilizes **antibodies** conjugated to fluorochromes as a means of identifying subsets of cells within a heterogenous sample. Purchasing from a vendor does not guarantee the functionality of the antibody. **Validation** must be carried out for all antibodies to allow **rigor and reproducibility** in your Flow Cytometry experiments.

Know Your Antibodies

Whenever possible, utilize **monoclonal or recombinant** antibodies for higher specificity. Due to the nature of production,  polyclonal antibodies will have more than just one paratope.

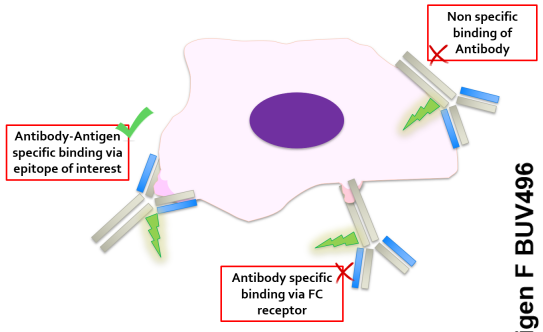
Methods of Binding

- Binding of the antibody to the antigen through the Fab is a specific method of binding via the epitope of interest.
- Antibodies can also specifically bind to cells via **FC mediated binding**. This results in false positives for your marker of interest and can be prevented with FC blocking. *(See FC Block Flow Post-it, Feb 2020)*
- Certain fluorochromes, i.e. Cyanine dyes, can bind to your cells via fluorochrome mediated **non-specific binding**. This can be prevented with commercially available blocker reagents.
- Oversaturation of your cells with antibody can cause non-specific staining and difficult to interpret results. This is avoided by carrying out an **antibody titration** on your cells of interest.

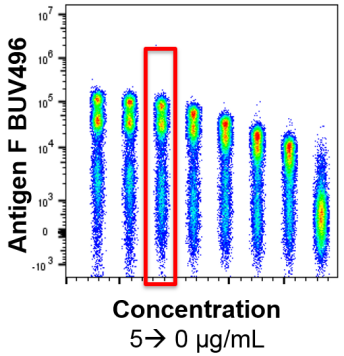
Controls

- Validate your antibody by testing it with the appropriate cell lines or tissue that you know do (**positive**) or do not (**negative**) express the specific protein (and isoform) that you are using it for. Transfected and/or KO cell lines are useful for these controls.
- Test for **cross reactivity**. Antibodies that are cross reactive across protein variants can result in data that is not reliable or reproducible.
- Unstimulated vs stimulated controls should be used when validating activation markers.

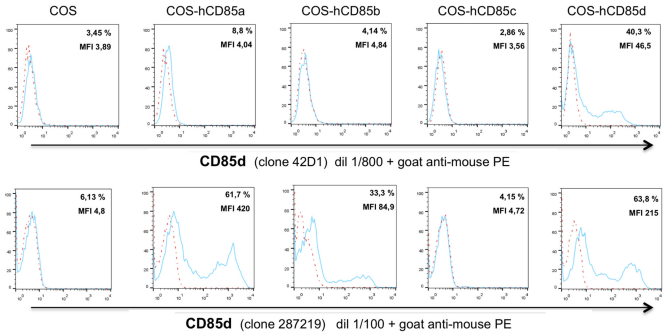
Methods of Ab Binding



Titrate



Cross Reactivity Testing



Kalina et. al Cytometry Part A 97A: 126–136