



Cornell University
College of Veterinary Medicine

Department of Biomedical Sciences

Flow Cytometry Core Facility

Yen Lab

Rev-0

October 15, 2015

Flow Cytometry Protocol

*Fluorescence Resonance Energy Transfer
(FRET) Cell Surface Cytometry Protocol*

ID#RM01

1. Select fluorochromes to be used and reference them using the Spectraviewer (<https://www.thermofisher.com/us/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html>) and be sure that the cytometer has the proper laser lines, filters, and mirrors available. We have had success with the Alexa 488/Alexa 594 FRET pair.
2. Determine whether there you will use a conjugated primary antibody or a primary antibody and a conjugated secondary antibody. In some cases, there may be commercially available conjugated primary antibodies available, but this generally only applies to the most popular antibodies. It is possible to conjugate primary antibodies on your own, however; this allows for the use of primary antibodies of the same isotype (e.g. two mouse antibodies) since secondary antibodies will detect any primary antibody made in a given animal (rabbit, mouse, goat, etc.).
3. To conjugate a primary antibody yourself, follow the antibody conjugation protocol.
4. Determine the samples to be run and label sample tubes.
 - a. For conjugated primary antibodies, make sure to have at least four samples: an unstained control, a control stained with the donor only (Alexa 488-conjugated primary), a control stained with the acceptor only (Alexa 594-conjugated primary), and a double stained sample (mix of both Alexa 488-conjugated primary and Alexa 594-conjugated primary).
 - b. For unconjugated primary antibodies, make sure to have at least nine samples: an unstained control, a sample stained with primary 1 + donor secondary, primary 1 + acceptor secondary, primary 1 + both secondaries, primary 2 + donor secondary, primary 2 + acceptor secondary, primary 2 + both secondaries, a sample with both secondaries only, and a FRET sample with both primaries and both secondaries. It may also be useful to include samples stained with nonspecific primaries (e.g. rabbit IgG or mouse IgG) in place of one or the other true primary antibodies as controls.
5. To prepare cells, spin down 1 million cells at 700 RPM for 5 min, decant the media, and resuspend and wash the pellet with 1 mL of phosphate-buffered saline (PBS). Next, spin down the cells again, and perform a second wash.
6. Prepare stock dilutions of all required primary antibody stains such that each tube will get 200 uL of stain in PBS. We have had success using 1.5-2 uL of antibody per 200 uL PBS, but you should consult the product information sheets of your antibodies and use the recommended concentrations. For samples to be stained with both primary antibodies, be sure to prepare a stock dilution containing both.
7. Spin down the cells and resuspend in 200 uL of antibody solution. Incubate at 37°C for 1 hour (we use our cell culture incubator).



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8. Spin down cells and perform two PBS washes as above.
 - a. If using conjugated primary antibodies, resuspend in 200 uL of PBS and read the samples using the LSR II or the FACS Aria II, based on laser lines required for the fluorochromes. If using the Alexa 488/Alexa 594 FRET pair, you will need to use the FACS Aria II.
 - b. If using conjugated secondary antibodies, prepare dilutions of secondary antibody in the same manner as the primaries, and resuspend each pellet in appropriate secondary antibody dilutions. Incubate at 37°C for 1 hour, then perform two more PBS washes, resuspend in 200 uL of PBS, and read the samples.