SIGNAL AMPLIFICATION FOR FLOW CYTOMETRY
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Often times, we are in the need of amplifying flow cytometry signal to be able to analyze expression of markers that are not at all abundant, or for which antibodies are not that good (e.g. surface CTLA4, SP1R1). Here are some validated tricks to do that.

MATERIALS
FACS Buffer: PBS 0.5%BSA
If needed: buffers for intracytoplasmic or intranuclear detection of antigens

For Method 2:
anti-PE antibody, Biotin conjugated: Biolegend clone PE001 (cat n. 408103)
anti-APC antibody, Biotin conjugated: Biolegend clone APC003 (cat. N. 408003)

METHOD 1: BASED ON SPECIES OF ORIGIN OF THE PRIMARY AB
(Recommended in case of intracellular / intranuclear antigens)

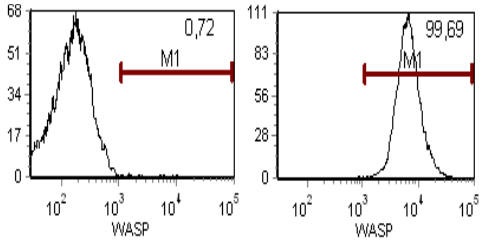
1. Perform primary staining using an antibody PRODUCED in species X and conjugated to fluorophore Y. IMPORTANT: include a sample stained with isotype-matched control antibody, also conjugated to fluorophore Y. Wash twice before step 2.

2. Do secondary staining with an antibody PRODUCED on species Z but REACTIVE to species X and conjugated to fluorophore Y. Wash twice before step 3.

3. Do tertiary staining with an antibody PRODUCED on species X but REACTIVE to species Z and conjugated to fluorophore Y.

4. If you need so, you can enhance signal further by performing additional rounds of staining as in step 2 and 3.

Example: Platelets were stained with anti-WASP non-conjugated and produced in Rabbit. Secondary staining was performed with goat anti-rabbit Ax488 and tertiary with rabbit anti-goat Ax488. Here is a comparison of WASP-KO vs. WT platelets.



METHOD 2: BASED ON FLUOROPHORE
(Might work on permeabilized cells if endogenous biotin is blocked)

1. Perform primary staining using an antibody conjugated to fluorophore Y. IMPORTANT: include a sample stained with isotype-matched control antibody, also conjugated to fluorophore Y. Wash twice before step 2.

2. Do secondary staining with an antibody REACTIVE to fluorophore Y, conjugated to BIOTIN. Wash twice before step 3.

3. Do tertiary staining with STREPTAVIDIN conjugated with fluorophore Y.

4. If you need so, you can enhance signal further by performing additional rounds of staining as in step 2 and 3.

Example: Treg were surface stained with anti-CTLA-4 APC. Secondary staining was performed with biotin anti-APC Abs, tertiary with SA-APC.