Institut für HIV Forschung
SOP #06-02 **(BS Juni 2016)**

Staining Compensation Beads

Reagents

Reagent	Vendor	Catalogue #
FACS Tubes	BD Falcon	352054
Live/Dead Fixable Aqua Dead Cell Stain Kit	Biolegend	423102
Antibodies used in assay	Biolegend/BD/eBioscience	
Anti-mouse Ig/Negative Control Compensation Particle Set	BD	552843
Anti-rat and Anti-hamster Ig/Negative Control Compensation Particle Set	BD	552845
ArC Amine Reactive Compensation Bead Kit	Biolegend	A10346

This protocol is for creating single stained, compensation controls for FACS analysis. These beads should be prepared the same day as analysis and NOT left overnight.

1. We will create conpensation control tubes that will each contain one antibody each. Therefore, label one FACS tube for each antibody used in your assay including one for the Live/Dead (viability) stain. Make sure you write the fluorochrome of each antibody on the tube and, if desired, the antibody used (e.g. CD3)
    a. Also include an Unstained control. This tube will contain only Negative control beads from BD and no antibody should be added to
2. Check which animal your antibodies are derived from. This information can be found on the product sheet or the manufacturer's website usually under "isotype" or "species" for that antibody. A list of commonly used lineage antibodies can be found on the server and it has this information
    a. The viability dyes (Live/Dead) are amine reactive and the species is irrelevant. These dyes require "ArC" beads and will be referred to as ArC beads separately throughout the rest of the protocol
3. The BD compensation beads are species specific (mouse, rat/hamster). For each compensation tube that was made in step 1, identify which compensation beads must be used for each one (mouse, rat/hamster, or ArC)
4. Retrieve the bead vials you require from the refrigerator in the B3 lab. They can be found in a box labeled "Beads"
5. Vortex all of the vials of beads prior to dispensing. Vortex them for about 5 seconds to ensure the beads are resuspended in solution
6. Add one drop of positive anti-mouse beads (large vial with blue cap) to all compensation tubes that will be stained with an antibody derived from a mouse
7. Add one drop of positive anti-rat/hamster beads (large vial with green cap) to all appropriate compensation tubes that will be stained with an antibody derived from a rat/hamster

8. Add **three** drops of positive ArC reactive (small vial with green cap) to your Live/Dead compensation tube
9. Add 1 drop of Negative Control beads (large vial with white cap) to all compensation tubes that will stained with either a mouse antibody OR rat/hamster antibody (add 2 drops to the unstained control)
    a. These beads can act as a negative control for both Anti-mouse Ig beads and Anti-rat/hamster Ig beads
    b. Note: the Arc Negative beads will be added to the Live/Dead compensation tube later
10. Add 2ml PBS to all compensation tubes (including the tube that contains ArC beads). Vortex them and spin them at 600xg, 5min
11. During the spin, take out the Live/Dead dye from the freezer.
12. After the compensation tubes are done spinning, aspirate the supernatant
    a. Though you cannot see the beads on the bottom, you have to trust that they are there. They will remain there after aspiration
13. Add 100ul PBS to each tube
14. Add 3ul of your Live/Dead dye to the compensation tube with ArC beads. Vortex the ArC compensation tube and incubate for 30 min in the dark. This tube will be treated separately from the rest. After the 30 min, go to step 21
15. Add the antibodies used in the assay to their respective tubes. Use the same dilution/amount per tube as you did in your assay. Each tube should only have **one** antibody added to it (except for the unstained control)
16. Incubate in the dark for 5 min
    a. The manufacturer protocol suggests 15-30 min incubation time, but 5 min is enough
17. Add 2ml PBS to tubes and spin them at 600xg, 5 min
18. Aspirate the supernatant
19. Add 150ul PBS to each tube and vortex
20. Place the tubes in a FACS rack and wrap it in aluminum foil. Store these at 4°C until analysis
21. After the 30 min incubation, add 2ml PBS to the ArC compensation tube and spin at 600xg, 5 min
22. Aspirate the supernatant
23. Vortex the ArC Negative beads (small vial with white cap) and add 3 drops to the ArC compensation tube
24. Add 100ul PBS to the tube and vortex, store with the other compensation tubes under the foil at 4°C until analysis