vFC™ Protocol 0.3 - Fluorescence Calibration using vCal™ Antibody Capture Beads

Objective

Calibrate the relevant fluorescence channels to report intensity in absolute units of antibodies bound per vesicle (ABV).

Materials

- vCal™ Antibody Capture Nanobeads (800 nm diameter polystyrene, $1x10^7$/mL)
  Note: Calibrated beads are available for each of mouse, rat, rabbit, and hamster antibodies.
- Fluorescent antibody conjugate(s) (10x)
- vFC™ Staining Buffer
- v-bottom plate or 0.65 mL microfuge tubes

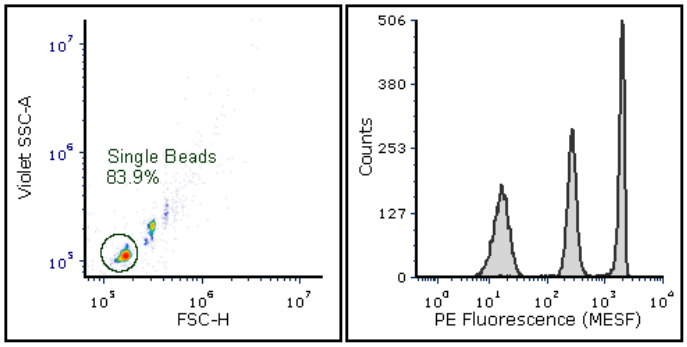
Procedure

1. Briefly vortex Capture Beads.
2. Place 40 ul vFC™ Staining Buffer in a v-bottom plate or 0.65 mL microfuge tube for each marker to be calibrated.
3. Add 5 uL of each fluorescent antibody conjugate to be calibrated to a different well
4. Add 5 uL of vCal™ Antibody Capture Nanobeads to each well and mix. Incubate for 1 hour at RT.
5. Wash beads by in 300 uL buffer by two cycles of filtration (<0.45 um pore) or centrifugation (10,000 xg, 5 min) and resuspension. Resuspend in buffer (100-300 uL) and measure at same fluorescence channel gains as for vFC™ analysis.

Data Analysis

1. Open the vCal™ Ab Capture Bead analysis layout using FCS Express or FCS Express Reader
2. Load the appropriate data files
3. Use the bivariate plot of scatter parameters (eg VSSC vs SSC) to identify and gate on the population of single beads
4. Use the univariate plot of marker fluorescence intensity in the appropriate channel to visually inspect the separation of three bead populations and set the markers to.
5. Use the FCS Express Channel Calibration Tools to generate a calibration file (Tools>Channel Calibration) to estimate the number of FL-mAbs bound per EV.

Example Data



vCal™ Mouse Antibody Capture Nanobeads stained with anti-CD235ab PE antibody.

vCal™ Mouse Antibody Capture Nanobeads stained with PE antibody conjugate.

Calibrated antibody binding capacities

(Lot# 181212):

Peak 1:	0 IgG molecules
Peak 2:	273 IgG molecules
Peak 3:	1982 IgG molecules