SCIF SOP for Flow Cytometer Standardization

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1) Set up experiment with all proper voltage parameters. Do compensation, etc. Run your samples and record them as normal. This experiment will serve as the baseline. Once all samples are run, you will turn off compensation for the next tubes you are about to record. You will use these voltage settings to generate your instrument baseline target values. Select QC beads to utilize and note the LOT#. Recommend you use CST beads, 6 peak beads, or some sort of multi-peak bead mixture, that is easy to prepare and highly reproducible. Make sure compensation is turned off before recording anything at this point. To determine your baseline target values, you will record and document the median fluorescence intensity (MFI) values or mode values on a distinct population peak of the beads that is on scale, which are gated on FSC vs SSC, and singlet beads. This will establish a reference point for the machine. Next time before running your compensation controls and experimental samples, the instrument will be required to hit the baseline target values by adjusting voltages for each channel with compensation turned off. If values greater than 10% in either direction, machine might need realignment/PM/adjustment. Create a new Global worksheet and rename it Standardization QC so you can record and track the MFIs. Gating on a bead peak near 10^5 is recommended. Make sure to note which peak it is. For example, if using 8-peak beads make sure you know which peak is your target population. This may be different for each channel.
2) When setting up your next experiment, you will have your target MFIs from your baseline. Duplicate an old experiment without data. Note the MFI target values from your baseline experiment. We suggest making a printout of these values to reference every time you perform an experiment. Make sure compensation is turned off for these QC tubes. Under the previous sample baseline tube, run the beads, and record the "preset" tube (before any voltage adjustments). Check these preset values to the baseline target values for each channel. If they are off by more than 10% you'll need to adjust voltages. For the next tube, adjust the voltages on FSC, SSC, and the appropriate PMTs to match the baseline target values. Once set, record a "match Baseline" tube after you hit the MFI target values. Under your compensation controls, make sure the voltage settings match those of the tube you just recorded on all parameters. The machine has been standardized at this point. You should not have to adjust voltages during the compensation setup step as the machine is back to its previous state. Begin checking and if good, record your comp controls.
3) With these values you just recorded and set, the machine will be standardized back to your original baseline. This does not control for variability in pipetting, antibody dilution, cell concentration. The more that these other variables stay stable, the assay will be highly reproducible, as a huge variable, the flow cytometer, has been standardized.
4) Record a tube of the beads (compensation turned off) as a final "post" tube with the settings at the end of the experiment, so you can check to see if the machine experienced any potential drift or issues during the run. Remember to make sure compensation is turned off for any QC tube involving the beads.
5) If you are running out of a lot of beads, you can reset your MFI target values by first normalizing the machine to the baseline values following the steps above. Make sure compensation is turned off. Once machine is normalized to baseline, record and note the new lot of beads under "reset target values" tube and document the new target MFI values for the peak of interest. For any future experiment, you can use these new MFI target values to standardize the machine accordingly back to its original baseline.

Recommended beads:

Spherotech: CAT# URCP-38-2K