UWCCC Flow Cytometry Laboratory
http://www.flowcytometry.wisc.edu

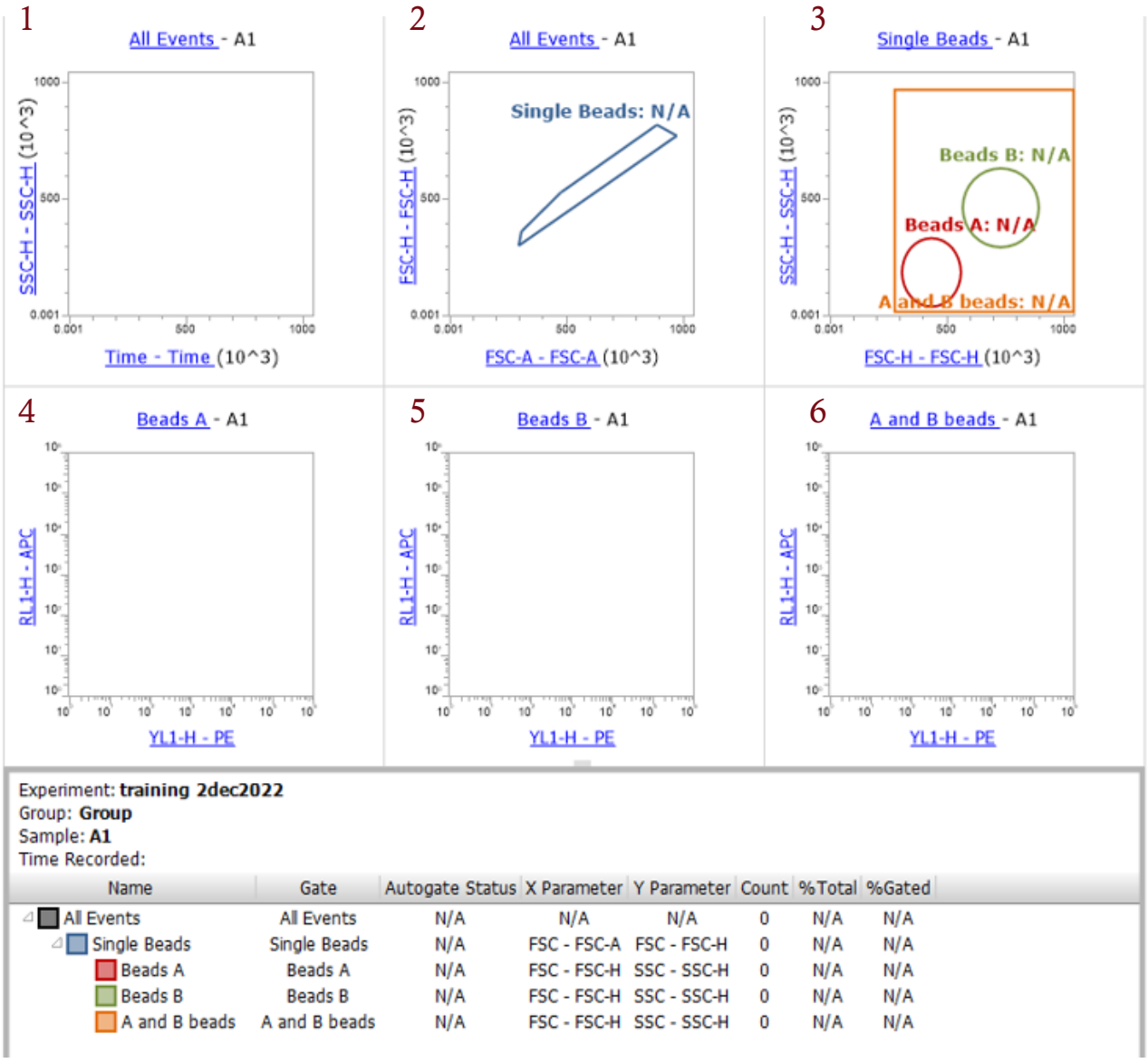
LEGENDplex Setup for Attune NxT

1111 Highland Ave
7016 WIMR
Madison, WI 53705
608.263.0313

Link to Official BioLegend Instructions

https://www.biolegend.com/Files/Images/BioLegend/legendplex/instructions/Setup-Procedure-for-Attune-NxT.pdf

Worksheet and Gating Illustration



Experiment: **training 2dec2022**
Group: **Group**
Sample: **A1**
Time Recorded:

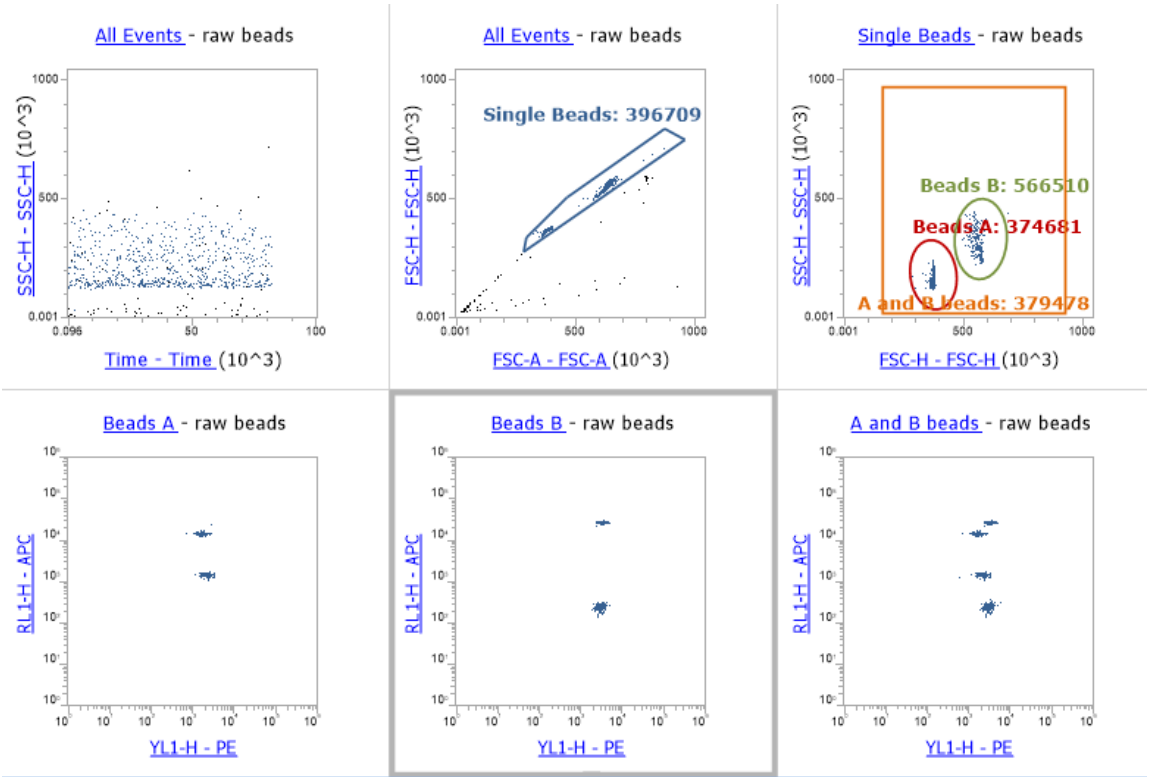
Name	Gate	Autogate Status	X Parameter	Y Parameter	Count	%Total	%Gated
All Events	All Events	N/A	N/A	N/A	0	N/A	N/A
Single Beads	Single Beads	N/A	FSC - FSC-A	FSC - FSC-H	0	N/A	N/A
Beads A	Beads A	N/A	FSC - FSC-H	SSC - SSC-H	0	N/A	N/A
Beads B	Beads B	N/A	FSC - FSC-H	SSC - SSC-H	0	N/A	N/A
A and B beads	A and B beads	N/A	FSC - FSC-H	SSC - SSC-H	0	N/A	N/A

Plots and Gates

Plot	X Axis	Y Axis	Show Population	Gate	Purpose
1	Time	SSC-H (or A)	All Events	-	Monitor stable flow
2	FSC-A	FSC-H	All Events	Single Beads	Eliminate aggregates
3	FSC-H (or A)	SSC-H (or A)	Single Beads	Beads A	Gate bead populations
				Beads B	Gate bead populations
				A and B beads	Gate bead populations
4	PE-H (or A)	APC-H (or A)	Beads A	-	Show analytes in A
5	PE-H (or A)	APC-H (or A)	Beads B		Show analytes in B
6	PE-H	APC-H	A and B beads		Show analytes on all beads

Setting Voltages

- Run Raw Beads at 25 µL/min
  - Adjust FSC and SSC voltages so both bead populations are visible
  - Suggest at least 200K difference in FSC MFIs of A beads and B beads for optimal separation without increasing spread (e.g. A population FSC median at 400K and B population FSC median at 600K)

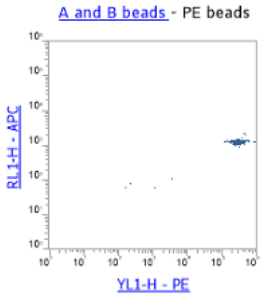


Experiment: _
Group: **Control Testing**
Sample: **raw beads**
Time Recorded: **14:18:12**

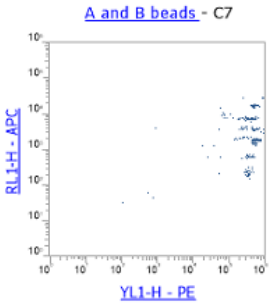
Name	Gate	X Parameter	Y Parameter	Count	%Total	%Gated	X Median
All Events	All Events	N/A	N/A	1,834	100.000	100.000	N/A
Single Beads	Single Beads	FSC - FSC-A	FSC - FSC-H	1,604	87.459	87.459	396,709
Beads A	Beads A	FSC - FSC-H	SSC - SSC-H	933	50.872	58.167	374,681
Beads B	Beads B	FSC - FSC-H	SSC - SSC-H	662	36.096	41.272	566,510
A and B beads	A and B beads	FSC - FSC-H	SSC - SSC-H	1,604	87.459	100.000	379,478

  - Adjust PE voltage so most beads are between 10^2 and 10^3 (as a starting point to put the negatives on scale, a little high in the figure above)
  - Adjust APC voltage so populations resolve and most are between $4x10^3$ and $7x10^5$ (a little low in the figure above)

- Run PE beads at 25 µL/min
    - Adjust PE voltage so PE signal is halfway between 10^5 and 10^6 (getting positives on scale in the linear range of the detector)
    - PE control beads exist only in the A population



- Run a control sample with the highest amount of analyte (Standard 7)
    - Fine-tune the PE voltage so the brightest population of beads has a PE signal is halfway between 10^5 and 10^6
    - The goal is to have the brightest sub-population be half a log back from the maximum signal, so that no beads run off scale in the experiment



- Ignore the FITC setup beads included with the kit

Recording Data

Record samples at 200 µL/minute

Set **Stop Options** to "3,000 events on Beads A" – to get enough events for analysis

Set **Record Events in** to "A and B beads" – to keep debris and aggregates out of data files