21 Color panel tested on the ZE5 by DG (10/30/20)

	Voltage
FSC-488	330
SSC-488	583

Rank	Fluorophore	Voltage	Notes
1	PE	540	
2	APC	600	
3	FITC	500	
4	BV421	535	
5	PECy7	650	
6	BV510	550	
7	PE/Dazzle594	575	
8	APC/Cy7	650	
9	BV605	600	
10	BV786	600	
11	BV711	600	
12	BV650	650	
13	BUV395	600	
14	BUV563	600	
15	BUV737	600	
16	BUV805	650	
17	PerCPCy5.5	580	Comp issue with PEDazzle 594 and autofluorescence, however, good if not using Dazzle594
18	APC/Fire 810	700	Not best signal and big comp with APC/Cy7
19	BUV661	600	Very bad spillover into APC
20	BUV496	600	Poor signal
21	AlexaFluor 700	640	Dim and bad spillover into PerCPCy5.5. BUV661, and BUV737 spilling in badly as well.

*This ranking is based on a combination of brightness on the ZE5, antibody availability, spillover into other channels, and fluorophore cost. There is no exact formula used, but rather David's qualitative assessment.

Method: B6 mouse LN cells (1x10^6 per well) were single stained at 1:200 with anti-CD4 (clone GK1.5) conjugated to each color listed above in FACS buffer for 15 minutes on ice, washed 2 times, and run in 96 well plates. Voltages were adjusted for good separation of the positive and negative peak, with positive signal ranging from 10^3-10^4. All antibodies were purchased from Biolegend or BD Biosciences.

Compensation Matrix

Reset | Area Height

Spillover

Fluorophores	FITC	PerCP-C...	BUV 661	BUV 737	BUV 805	BUV 395	BUV 563	BUV 496	BV 650	BV 711	BV 786	BV 421	BV 605	BV 510	PE-Cy7	PE-Dazzl...	PE	Alexa 700	APC-Cy7	APC-Fire...	APC
FITC	1	0.00%	0.00%	0.00%	0.00%	0.00%	1.25%	3.61%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.25%	0.00%	0.00%	0.00%	0.00%
PerCP-C...	3.23%	1	4.84%	16.54%	0.00%	0.00%	3.89%	0.16%	1.86%	11.61%	0.05%	0.00%	4.65%	0.00%	2.63%	63.50%	19.65%	1.71%	0.10%	0.10%	2.79%
BUV 661	0.07%	6.42%	1	0.95%	0.15%	0.04%	8.72%	1.51%	13.73%	1.79%	0.09%	0.02%	7.60%	2.50%	0.04%	1.58%	0.53%	0.18%	0.16%	0.23%	4.40%
BUV 737	0.01%	2.61%	15.82%	1	1.78%	0.00%	0.69%	0.06%	1.96%	13.76%	4.20%	0.00%	1.12%	0.26%	1.27%	0.13%	0.02%	0.56%	0.59%	0.02%	0.61%
BUV 805	0.01%	1.29%	7.63%	58.15%	1	0.01%	0.22%	0.04%	0.90%	9.52%	23.59%	0.00%	0.56%	0.12%	7.27%	0.04%	0.01%	0.19%	3.79%	4.26%	0.22%
BUV 395	0.40%	0.77%	12.55%	22.78%	40.05%	1	20.89%	17.15%	0.23%	0.00%	0.44%	0.13%	0.29%	0.00%	0.33%	0.18%	0.13%	0.00%	0.00%	0.42%	0.18%
BUV 563	0.81%	0.35%	0.29%	0.41%	0.71%	0.28%	1	16.74%	0.14%	0.18%	0.21%	0.24%	2.26%	12.32%	0.32%	0.83%	8.01%	0.40%	0.13%	0.25%	0.11%
BUV 496	2.63%	1.29%	1.72%	2.86%	5.18%	3.61%	3.11%	1	0.38%	0.93%	1.02%	3.11%	0.48%	26.08%	0.71%	0.39%	0.28%	1.47%	0.71%	0.67%	0.41%
BV 650	0.10%	26.71%	14.25%	0.05%	0.00%	0.00%	0.58%	0.57%	1	11.19%	0.42%	0.08%	53.33%	13.84%	0.04%	5.73%	1.81%	0.22%	0.30%	0.53%	11.39%
BV 711	0.01%	19.81%	3.07%	7.89%	0.02%	0.00%	0.04%	0.04%	23.75%	1	4.17%	0.01%	11.56%	2.97%	0.43%	1.07%	0.30%	3.70%	0.14%	0.05%	1.86%
BV 786	0.00%	7.60%	0.75%	6.90%	3.42%	0.00%	0.02%	0.01%	6.12%	44.48%	1	0.00%	3.89%	0.95%	17.27%	0.23%	0.03%	1.18%	6.65%	6.21%	0.55%
BV 421	0.02%	0.03%	0.08%	0.15%	0.33%	0.15%	0.14%	0.12%	5.42%	13.42%	9.66%	1	4.66%	0.07%	0.01%	0.01%	0.01%	0.04%	0.02%	0.02%	0.01%
BV 605	0.53%	0.00%	0.50%	0.00%	0.00%	0.00%	2.40%	2.19%	18.55%	0.25%	0.29%	0.41%	1	32.60%	0.16%	16.21%	7.14%	0.07%	0.00%	0.00%	0.09%
BV 510	4.17%	0.21%	0.00%	0.18%	0.00%	0.00%	0.58%	23.24%	0.70%	1.79%	1.56%	11.87%	0.62%	1	0.00%	0.00%	0.07%	0.25%	0.00%	0.00%	0.00%
PE-Cy7	0.00%	4.01%	1.11%	0.99%	0.33%	0.00%	0.04%	0.01%	0.30%	0.86%	0.72%	0.00%	0.63%	0.00%	1	2.16%	0.39%	0.52%	8.01%	4.75%	0.90%
PE-Dazzl...	0.00%	0.01%	0.92%	0.01%	0.03%	0.00%	12.77%	0.02%	1.35%	0.01%	0.01%	0.00%	19.92%	0.00%	0.68%	1	37.19%	0.04%	0.02%	0.01%	0.13%
PE	0.00%	0.01%	0.01%	0.01%	0.01%	0.00%	27.55%	0.01%	0.01%	0.01%	0.01%	0.00%	7.37%	0.00%	1.77%	18.11%	1	0.03%	0.01%	0.00%	0.00%
Alexa 70...	0.00%	35.43%	95.65%	91.10%	0.25%	0.00%	0.00%	0.00%	10.94%	53.07%	0.68%	0.00%	0.02%	0.00%	1.42%	0.06%	0.00%	1	9.42%	4.80%	63.72%
APC-Cy7	0.00%	6.99%	14.33%	36.84%	9.26%	0.00%	0.00%	0.00%	1.54%	11.85%	7.45%	0.00%	0.01%	0.01%	20.55%	0.01%	0.00%	20.32%	1	25.20%	10.94%
APC-Fire...	0.10%	3.64%	6.49%	22.41%	16.85%	0.00%	0.12%	0.00%	0.88%	6.16%	5.21%	0.00%	0.00%	0.00%	11.35%	0.04%	0.00%	11.53%	55.71%	1	6.36%
APC	0.00%	9.92%	114.61%	0.24%	0.00%	0.00%	0.00%	0.00%	12.04%	1.76%	0.02%	0.00%	0.02%	0.00%	0.02%	0.07%	0.00%	1.14%	2.99%	5.12%	1

**Table reads as Fluorophore on interest in the columns (along the top) and spillover into the other channels of interest in the rows. Eg. you can see BUV661 spills very strongly into the Alexa700 (95%) and APC channels (114%).