

Essentials for Multicolor Panel Building

Fluorescent Dye	Laser Line, nm	Max Ex, nm	Max Em, nm	Relative Brightness
StarBright UltraViolet 400	355	335	394	
Brilliant Ultraviolet 395	355	348	395	
StarBright UltraViolet 445	355	347	440	
Brilliant Ultraviolet 496	355	348	496	
StarBright UltraViolet 510	355	340	513	
Brilliant Ultraviolet 563	355	348	563	
StarBright UltraViolet 575	355	340	569	
StarBright UltraViolet 605	355	340	609	
Brilliant Ultraviolet 615	355	350	616	
Brilliant Ultraviolet 661	355	348	661	
StarBright UltraViolet 665	355	340	669	
Brilliant Ultraviolet 737	355	348	737	
StarBright UltraViolet 740	355	344	743	
StarBright UltraViolet 795	355	340	792	
Brilliant Ultraviolet 805	355	348	805	
DyLight 405	405	400	420	
Brilliant Violet 421	405	407	421	
StarBright Violet 440	405	383	436	
Super Bright 436	405	414	436	
eFluor 450	405	405	445	
BD Horizon V450	405	404	448	
Pacific Blue	405	401	452	
Brilliant Violet 480	405	436	478	
StarBright Violet 475	405	406	479	
BD Horizon V500	405	415	500	
Brilliant Violet 510	405	405	510	
StarBright Violet 515	405	402	516	
StarBright Violet 570	405	404	571	
Brilliant Violet 570	405	407	574	
Super Bright 600	405	414	600	
Brilliant Violet 605	405	407	602	
StarBright Violet 610	405	403	607	
Super Bright 645	405	414	645	
Brilliant Violet 650	405	407	650	
StarBright Violet 670	405	401	667	
Super Bright 702	405	414	702	
Brilliant Violet 711	405	407	711	
StarBright Violet 710	405	402	713	
StarBright Violet 760	405	403	754	
StarBright Violet 790	405	402	782	
Brilliant Violet 786	405	407	786	
Brilliant Blue 515	488	490	515	
DyLight 488	488	493	518	
A488	488	493	519	
KIRAVIA Blue 520	488	488	520	
FITC	488	490	525	
PerCP	488	490	675	
Brilliant Blue 700	488	485	693	
PerCP-Cy5.5	488	490	695	
StarBright Blue 700	488	473	703	
DyLight 550	561	562	576	
PE	488	561	496/562	578
PE-eFluor 610	488	561	496/562	607
PE/Dazzle 594	488	561	496/562	610
PE/Fire 640	488	561	496/562	639
PE-A647	488	561	496/562	667
PE-Cy5	488	561	496/562	667
PE-Cy5.5	488	561	496/562	695
PE/Fire 700	488	561	496/562	695
PE-A750	488	561	496/562	779
PE-Cy7	488	561	496/562	785
e-Fluor 660	640	633	660	
APC	640	650	661	
A647	640	650	665	
Cy5	640	649	670	
DyLight 650	640	654	673	
APC-R700	640	652	704	
R718	640	695	718	
A700	640	702	723	
APC-eFluor 780	640	650	780	
APC-Cy7	640	650	785	
APC/Fire 750	640	650	787	
APC/Fire 810	640	650	807	

Abbreviations: 7-ADD, 7-Aminoactinomycin D; Axxx, Alexa Fluor; APC, allophycocyanin; Cy, Cyanine; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; FSC, forward scatter; PB, Pacific Blue; PE, phycoerythrin; PerCP, peridinin chlorophyll; PI, propidium iodide; SBV, StarBright Violet; SSC, side scatter.

Fluorescent Dye Brightness

Fluorescent dye brightness depends on how many photons a dye emits when excited by a laser. Other factors influencing the brightness are the laser power, instrument configuration, and detectors. Brighter fluorescent dyes will generally give better separation between the negative and positive fraction in your sample.

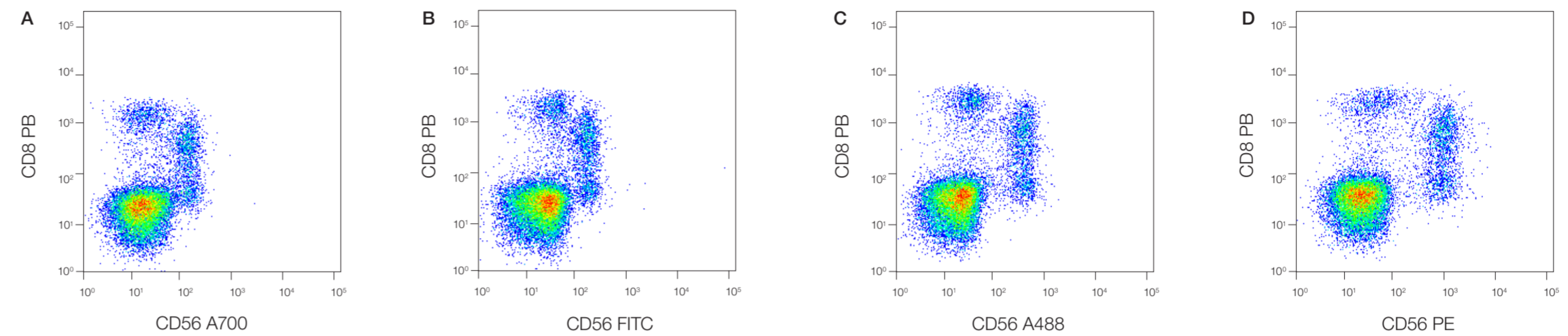


Fig. 1. CD8 and CD56 staining of human blood. The CD56⁺ and CD56⁻CD8⁺ can be more easily separated using brighter fluorescent dyes such as PE compared to dim fluorescent dyes like A700. A, A700; B, FITC; C, A488; D, PE.

Relative Antigen Density

Not all antigens are expressed at the same level on a cell surface. Match bright fluorescent dyes with low expressing markers and dim fluorescent dyes with highly expressed markers.

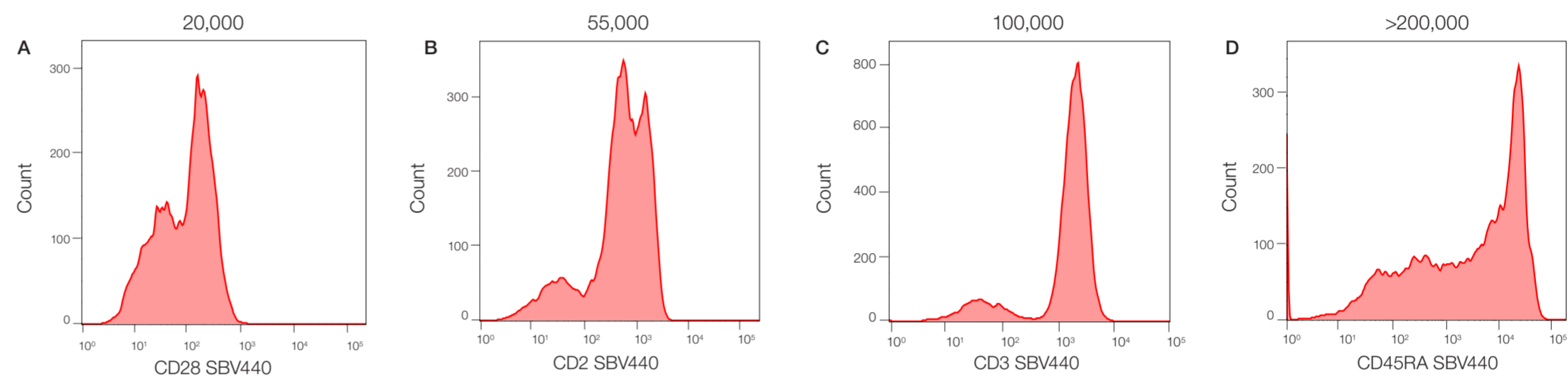


Fig. 2. CD28, CD2, CD3, and CD45RA staining of human blood. Human peripheral blood was stained with A, CD28 SBV440; B, CD2 SBV440; C, CD3 SBV440; and D, CD45RA SBV440. Low abundance proteins will appear dimmer (CD28) than high abundance proteins (CD45RA).

Antibody Titration

Careful titration of your antibodies will give you the best staining with the minimum background and thus improve the separation of your positive and negative populations (the staining index).

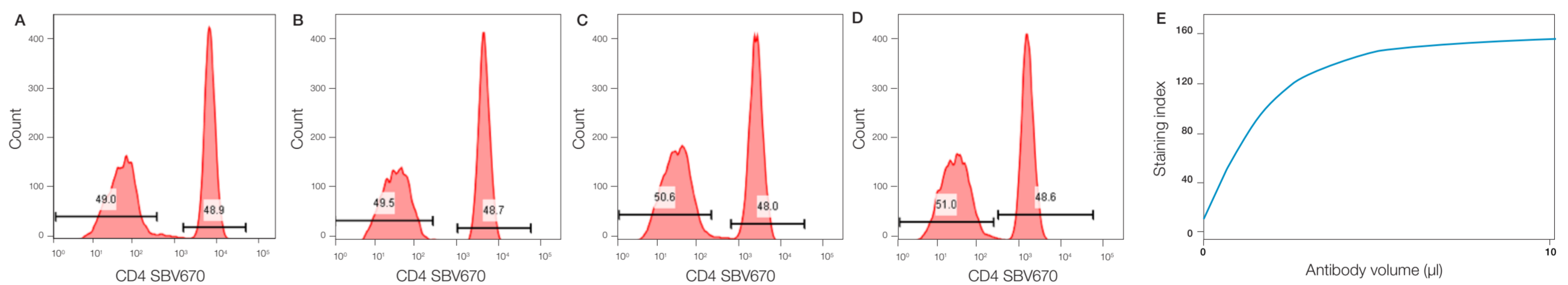


Fig. 3. Titration of CD4 on human blood. Human peripheral blood was stained with CD4 at increasing dilution. Optimal staining is identified by maximal separation of the negative and positive populations which was obtained using the antibody at 5 µl (1/2) dilution.

Viability Dyes

To improve your data, use a viability dye to exclude dead cells rather than forward and side scatter.

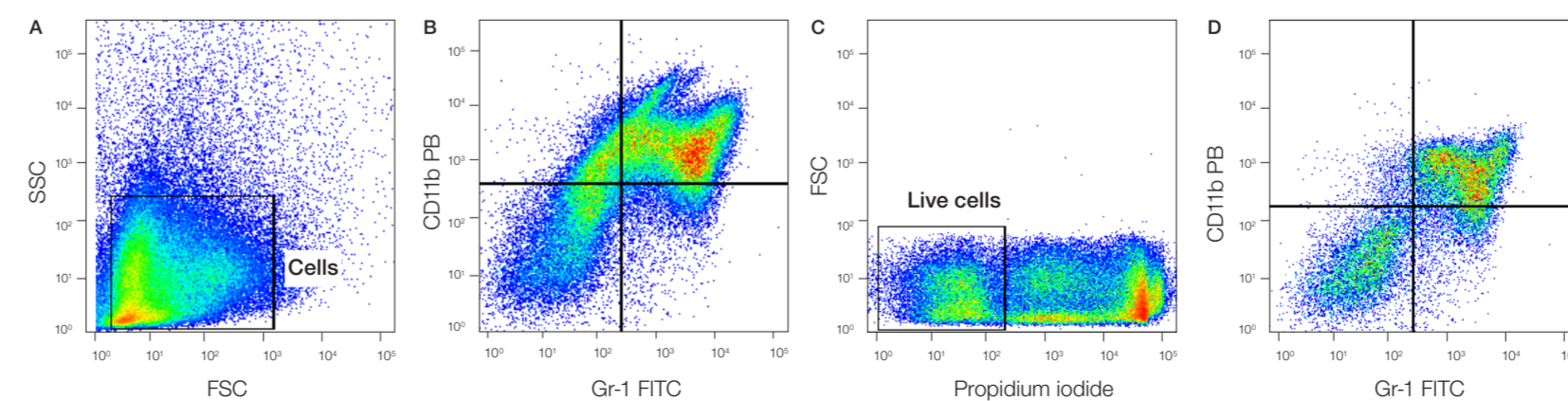


Fig. 4. Use of a viability dye on murine bone marrow. A and B, forward and side scatter may not be sufficient to remove dead cells from your analysis. C and D, dead cell exclusion, using a viability dye, can allow easier identification of positive and negative cell populations during data analysis.

Viability Dye	Laser Line, nm	Max Ex, nm	Max Em, nm
VivaFix 353/442	355	353	442
DAPI	355	405	359
PI	355	488	561
VivaFix 410/450	405	410	450
VivaFix 408/512	405	408	512
VivaFix 398/550	405	398	550
VivaFix 498/521	488	498	521
7-AAD	488	561	546
VivaFix 547/573	561	547	573
VivaFix 583/603	561	583	603
VivaFix 649/660	640	649	660