Essentials for Multicolor Panel Building

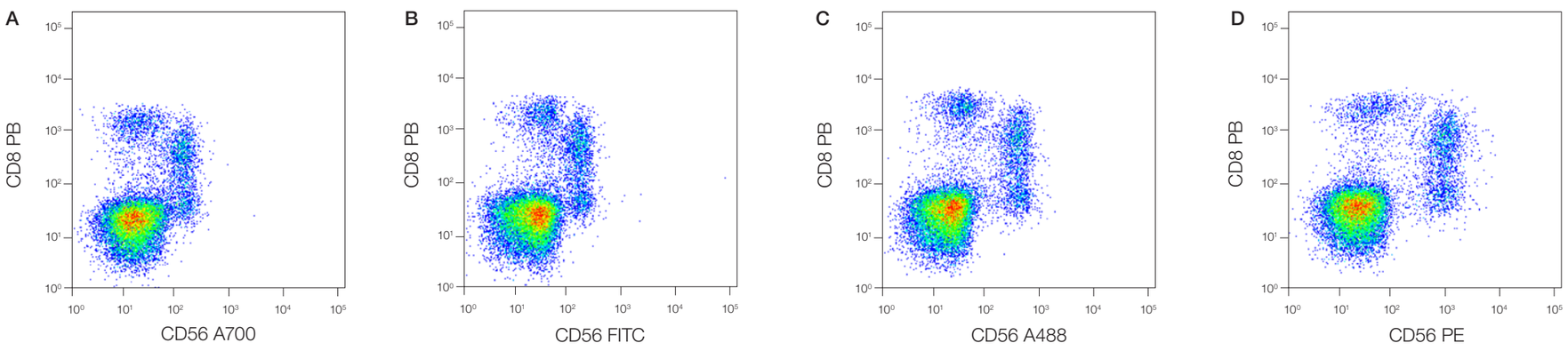
Fluorescent Dye	Laser Line, nm	Max Ex, nm	Max Em, nm	Relative Brightness
StarBright™ UltraViolet 400	355	335	394	
BD Horizon Brilliant Ultraviolet 395	355	348	395	
StarBright UltraViolet 445	355	347	440	
BD Horizon Brilliant Ultraviolet 496	355	348	496	
StarBright UltraViolet 510	355	340	513	
BD Horizon Brilliant Ultraviolet 563	355	348	563	
StarBright UltraViolet 575	355	340	569	
StarBright UltraViolet 605	355	340	609	
BD Horizon Brilliant Ultraviolet 615	355	350	616	
BD Horizon Brilliant Ultraviolet 661	355	348	661	
StarBright UltraViolet 665	355	340	669	
BD Horizon Brilliant Ultraviolet 737	355	348	737	
StarBright UltraViolet 740	355	344	743	
StarBright UltraViolet 795	355	340	792	
BD Horizon Brilliant Ultraviolet 805	355	348	805	
DyLight 405	405	400	420	
BD Horizon 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	
BD Horizon Brilliant Violet 480	405	436	478	
StarBright Violet 475	405	405	479	
BD Horizon V500	405	415	500	
BD Horizon Brilliant Violet 510	405	405	510	
StarBright Violet 515	405	402	516	
StarBright Violet 570	405	402	571	
BD Horizon Brilliant Violet 570	405	404	574	
Super Bright 600	405	414	600	
BD Horizon Brilliant Violet 605	405	407	602	
StarBright Violet 610	405	403	607	
Super Bright 645	405	414	645	
BD Horizon Brilliant Violet 650	405	407	650	
StarBright Violet 670	405	401	667	
Super Bright 702	405	414	702	
BD Horizon Brilliant Violet 711	405	407	711	
StarBright Violet 710	405	402	713	
StarBright Violet 760	405	403	754	
StarBright Violet 790	405	402	782	
BD Horizon Brilliant Violet 786	405	407	786	
BD Horizon Brilliant Blue 515	488	490	515	
DyLight 488	488	493	518	
A488	488	495	519	
KIRAVIA Blue 520	488	488	520	

Fluorescent Dye	Laser Line, nm	Max Ex, nm	Max Em, nm	Relative Brightness
FITC	488	490	525	
StarBright Blue 580	488	475	582	
StarBright Blue 615	488	475	612	
StarBright Blue 675	488	476	675	
PerCP	488	490	675	
BD Horizon Brilliant Blue 700	488	485	693	
PerCP-Cy5.5	488	490	695	
StarBright Blue 700	488	473	703	
StarBright Blue 765	488	476	764	
BD Horizon RealBlue 780	488	488	780	
StarBright Blue 810	488	477	802	
DyLight 550	561	562	576	
PE	488 561	496/546	578	
StarBright Yellow 575	561	548	579	
StarBright Yellow 605	561	572	606	
PE-eFluor 610	488 561	496/546	607	
PE-Dazzle 594	488 561	496/546	610	
PE-Fire 640	488 561	496/546	639	
PE-A647	488 561	496/546	667	
PE-Cy5	488 561	496/546	667	
StarBright Yellow 665	561	554	670	
PE-Cy5.5	488 561	496/546	695	
PE-Fire 700	488 561	496/546	695	
StarBright Yellow 720	561	549	719	
PE-A750	488 561	496/546	779	
PE-Cy7	488 561	496/546	785	
StarBright Yellow 800	561	549	788	
eFluor 660	640	633	660	
APC	640	650	661	
A647	640	650	665	
StarBright Red 670	640	653	666	
Cy5	640	649	670	
DyLight 650	640	654	673	
APC-R700	640	652	704	
StarBright Red 715	640	638	712	
R718	640	695	718	
A700	640	702	723	
StarBright Red 775	640	653	778	
APC-eFluor 780	640	650	780	
APC-Cy7	640	650	785	
APC-Fire 750	640	650	787	
APC-Fire 810	640	650	807	
StarBright Red 815	640	654	811	

Axxx, Alexa Fluor; APC, allophycocyanin; Cy, cyanine; FITC, fluorescein isothiocyanate; Max Em, maximum emission; Max Ex, maximum excitation; PE, phycoerythrin; PerCP, peridinin chlorophyll.

Fluorescent Dye Brightness

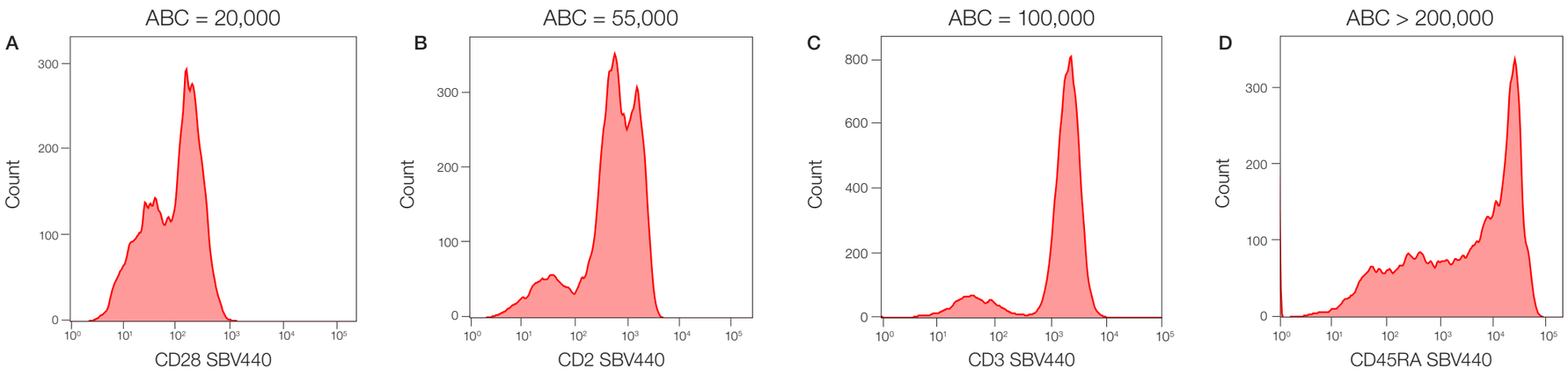
Fluorescent dye brightness depends on how many photons a dye emits when excited by a laser. Other factors influencing the brightness are the laser power, instrument configuration, and detectors. Brighter fluorescent dyes will generally give better separation between the negative and positive fractions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



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. Axxx, Alexa Fluor; FITC, fluorescein isothiocyanate; PB, Pacific Blue; PE, phycoerythrin.

Relative Antigen Density

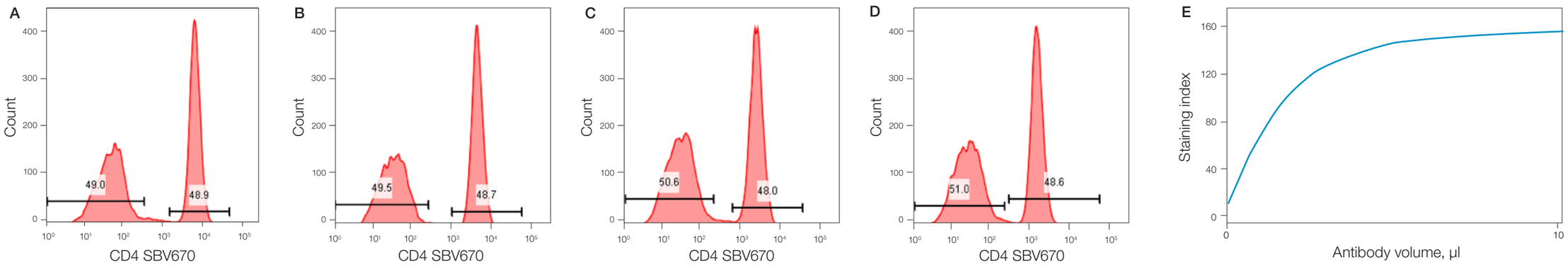
Not all antigens are expressed at the same level on a cell surface. Match bright fluorescent dyes with poorly expressed markers and dim fluorescent dyes with highly expressed markers.



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). ABC, antibody binding capacity; SBV, StarBright Violet.

Antibody Titration

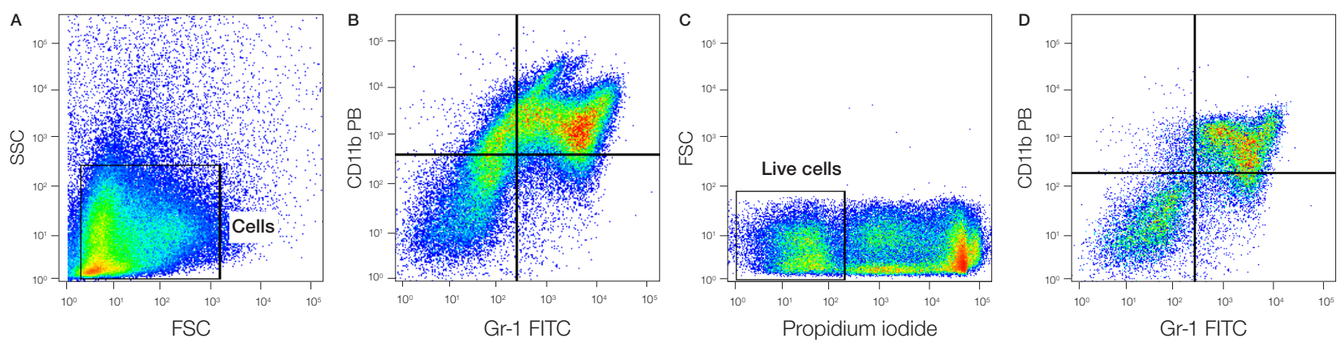
Careful titration of your antibodies will give you the best staining with the minimum background and thus improve the separation of your positive and negative populations (the stain index).



Titration of CD4 on human blood. Human peripheral blood was stained with CD4 at increasing dilution. **A**, 10 µl antibody; **B**, 5 µl antibody (1:2 dilution); **C**, 2.5 µl antibody (1:4 dilution); **D**, 1.25 µl antibody (1:8 dilution). **E**, optimal staining is determined by the stain index, or the point at which there is maximal separation of the negative and positive populations. The optimal amount of antibody in this case is 5 µl (1:2 dilution). SBV, StarBright Violet.

Viability Dyes

Improve your data by using a viability dye instead of forward and side scatter to exclude dead cells.



Use of a viability dye on murine bone marrow. A–B, forward and side scatter may not be sufficient to remove dead cells from your analysis. **C–D**, dead cell exclusion, using a viability dye, can allow easier identification of positive and negative cell populations during data analysis. FITC, fluorescein isothiocyanate; FSC, forward scatter; PB, Pacific Blue; SSC, side scatter.

Viability Dye	Laser Line, nm	Max Ex, nm	Max Em, nm
VivaFix 353/442	355	353	442
DAPI	355 405	359	461
PI	355 488 561	490	617
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	647
VivaFix 547/573	561	547	573
VivaFix 583/603	561	583	603
VivaFix 649/660	640	649	660

7-AAD, 7-aminoactinomycin D; DAPI, 4'-6-diamidino-2-phenylindole; Max Em, maximum emission; Max Ex, maximum excitation; PI, propidium iodide.

Visit **bio-rad-antibodies.com/flow** to view our full range of products optimized for flow cytometry.

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