Essentials for Multicolor Panel Building

Fluorophore	Laser Line, nm	Max Ex, nm	Max Em, nm	Relative Brightness
BD Horizon Brilliant Ultraviolet 395	355	348	395	
Alexa Fluor 350	355	340	440	
BD Horizon Brilliant Ultraviolet 496	355	348	496	
BD Horizon Brilliant Ultraviolet 563	355	348	563	
BD Horizon Brilliant Ultraviolet 661	355	348	661	
BD Horizon Brilliant Ultraviolet 737	355	348	737	
BD Horizon Brilliant Ultraviolet 805	355	348	805	
DyLight 405	405	400	420	
Alexa Fluor 405	405	401	420	
BD Horizon Brilliant Violet 421	405	407	421	
eFluor 450	405	405	445	
BD Horizon V450	405	404	448	
Super Bright 436	405	414	436	
Pacific Blue	405	401	452	
BD Horizon Brilliant Violet 480	405	436	478	
BD Horizon V500	405	415	500	
BD Horizon Brilliant Violet 510	405	405	510	
BD Horizon Brilliant Violet 570	405	407	574	
Super Bright 600	405	414	600	
BD Horizon Brilliant Violet 605	405	407	602	
Super Bright 645	405	414	645	
BD Horizon Brilliant Violet 650	405	407	650	
Super Bright 702	405	414	702	
BD Horizon Brilliant Violet 711	405	407	711	
BD Horizon Brilliant Violet 786	405	407	786	
BD Horizon Brilliant Blue 515	488	490	515	
DyLight 488	488	493	518	
Alexa Fluor 488	488	495	519	
FITC	488	490	525	
PerCP	488	490	675	
BD Horizon Brilliant Blue 700	488	485	693	
PerCP-Cy5.5	488	490	695	
DyLight 550	561	562	576	
PE	488 561	496/546	578	
PE-eFluor 610	488 561	496/546	607	
PE/Dazzle 594	488 561	496/546	610	
PE-Alexa Fluor 647	488 561	496/546	667	
PE-Cy5	488 561	496/546	667	
PE-Cy5.5	488 561	496/546	695	
PE-Alexa Fluor 750	488 561	496/546	779	
PE-Cy7	488 561	496/546	785	
eFluor 660	640	633	660	
APC	640	650	661	
Alexa Fluor 647	640	650	665	
Cy5	640	649	670	
DyLight 650	640	654	673	
Alexa Fluor 700	640	702	723	
APC-eFluor 780	640	650	780	
APC-Cy7	640	650	785	
APC/Fire 750	640	650	787	

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Abbreviations: Axxx, Alexa Fluor; APC, allophycocyanin; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride; FITC, fluorescein isothiocyanate; PB, Pacific Blue; PE, phycoerythrin; PerCP, peridinin chlorophyll; PI, propidium iodide.

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Fluorophore Brightness

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

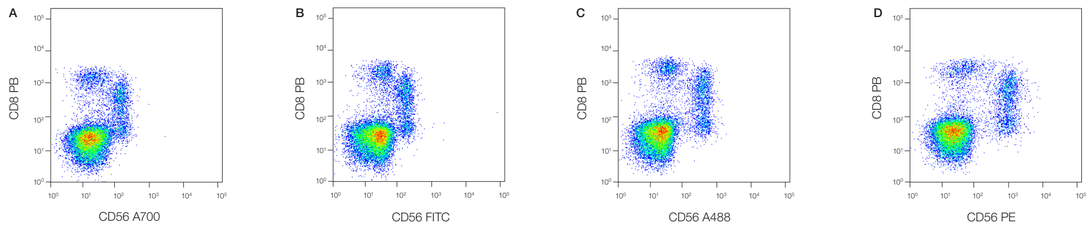


Fig. 1. CD8 and CD56 staining of human blood. The $CD56^+$ and $CD56^+CD8^+$ can be more easily separated using brighter fluorophores such as PE compared to dim fluorophores such as A700. **A**, A700; **B**, FITC; **C**, A488; **D**, PE.

Relative Antigen Density

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

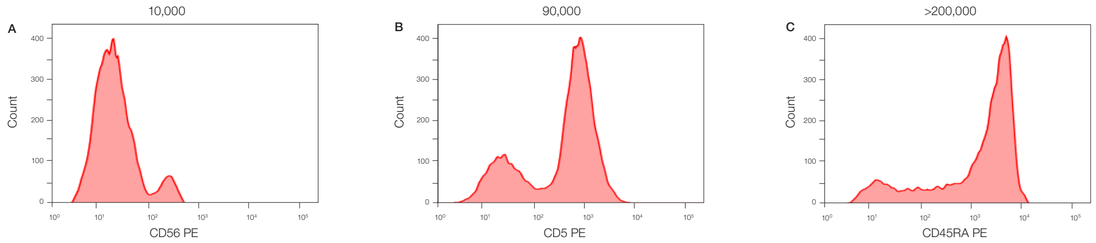


Fig. 2. CD56, CD5 and CD45RA staining of human blood. Human peripheral blood was stained with **A**, CD56 PE; **B**, CD5 PE and **C**, CD45RA PE. Low abundance proteins will appear dimmer (CD56) than high abundance proteins (CD45RA).

Antibody Titration

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

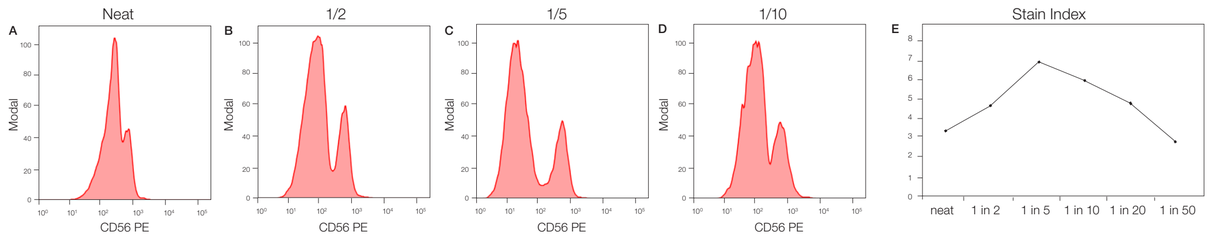
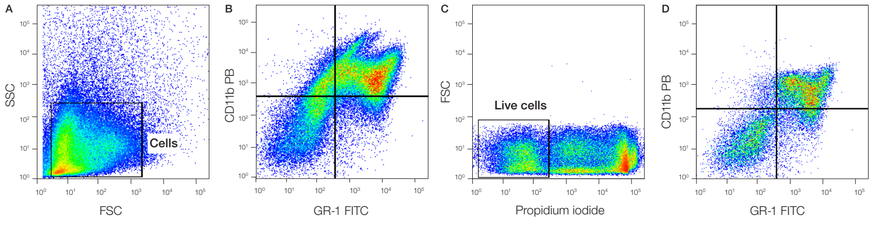


Fig. 3. Titration of CD56 on human blood. A-D, human peripheral blood was stained with CD56 at increasing dilution as shown. **E**, the stain index was calculated with the optimal separation of the positive and negative populations at 1/5 dilution of the antibody stock solution.

Viability Dyes

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



Viability Dye	Laser Line, nm	Max Ex, nm	Max Em, nm
VivaFix™ 353/442	355	353	442
DAPI	355 488	359	461
PI	355 488 561	490	617
VivaFix 410/450	405	410	450
VivaFix 408/512	405	408	512
VivaFix 398/550	405	389	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

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

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