invitrogen

Flow cytometry

Spectral flow cytometry assays and reagents

Conjugated antibodies

RNA flow cytometry

Invitrogen™ fluorescent probes and reagents are suitable for all cytometry instrumentation, including spectral flow cytometers. Many previously incompatible labeling dyes and functional reagents can now be used together in your expanded multicolor application. For example, in Figure 1, the significant overlap of Invitrogen™ PerCP and eBioscience™ PerCP-eFluor™ 710 dyes has generally disallowed their concomitant use. However, spectral cytometers can easily separate these fluorescence signals by identifying subtle differences in their overall spectra (red boxes in Figure 1). Now, both common and uncommon, dim and bright, and functional and nonfunctional fluorescence assays and reagents are increasingly feasible to combine.

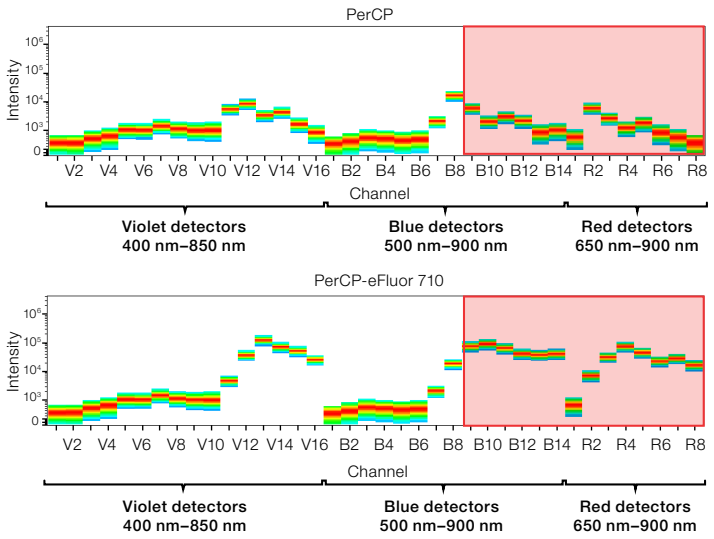

Figure 1. Comparison of a large protein molecule and its tandem dye–conjugated counterpart. PerCP dye–positive cells (top graph) compared to PerCP-eFluor 710 dye–positive cells (bottom graph) analyzed on a 3-laser spectral cytometer*. The unique patterns in the far-red channels allow for the two molecules to be discriminated.

Invitrogen reagents in a 20-color panel

Polychromatic flow cytometry has become an essential tool for studying immunology and has helped advance the field in both research and translational settings. Invitrogen fluorescent antibodies can be relied upon while undertaking complex panel design and approaches to immunophenotyping. Table 1 shows an example of a 20-color spread matrix that was generated with Invitrogen labeling reagents, to demonstrate their use on a 3-laser spectral flow cytometer provided by Cytek Biosciences.

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Table 1. Invitrogen™ antibodies for a 20-color panel.

Antibody conjugates for flow cytometry		
Violet 405 nm	**Blue 488 nm**	**Red 633 nm**
CD127 (eBioRDR5) Super Bright 436	CD45RA (HI100) FITC	CD27 (O323) APC
CD16 (eBioCB16 (CB16)) eFluor 450	CD3 (UCHT1) Alexa Fluor 532	CD57 (TB01 (TBO1)) eFluor 660
CD4 (RPA-T4) eFluor 506	CD28 (CD28.2) PE	CD14 (61D3) Alexa Fluor 700
CD8 (3B5) Pacific Orange	CD25 (CD25-4E3) PE-eFluor 610	CD38 (HIT2) APC-eFluor 780
IgD (IA6-2) Super Bright 600	CD95 (DX2) PE-Cyanine5	
CD56 NCAM (TULY56) Super Bright 645	CD19 (SJ25C1) PE-Cyanine5.5	
HLA-DR (LN3) Super Bright 702	TCR gamma/delta (B1.1) PerCP-eFluor 710	
CD197 (CCR7) (3D12) Super Bright 780	CD279 (PD1) (eBioJ105 (J105)) PE-Cyanine7	

Note: All dyes used in this immunophenotyping experiment are from the spread matrix (Table 1), with eFluor 660 dye replacing Alexa Fluor 647 dye. All data were generated on the 3-laser spatially offset spectral flow cytometer.

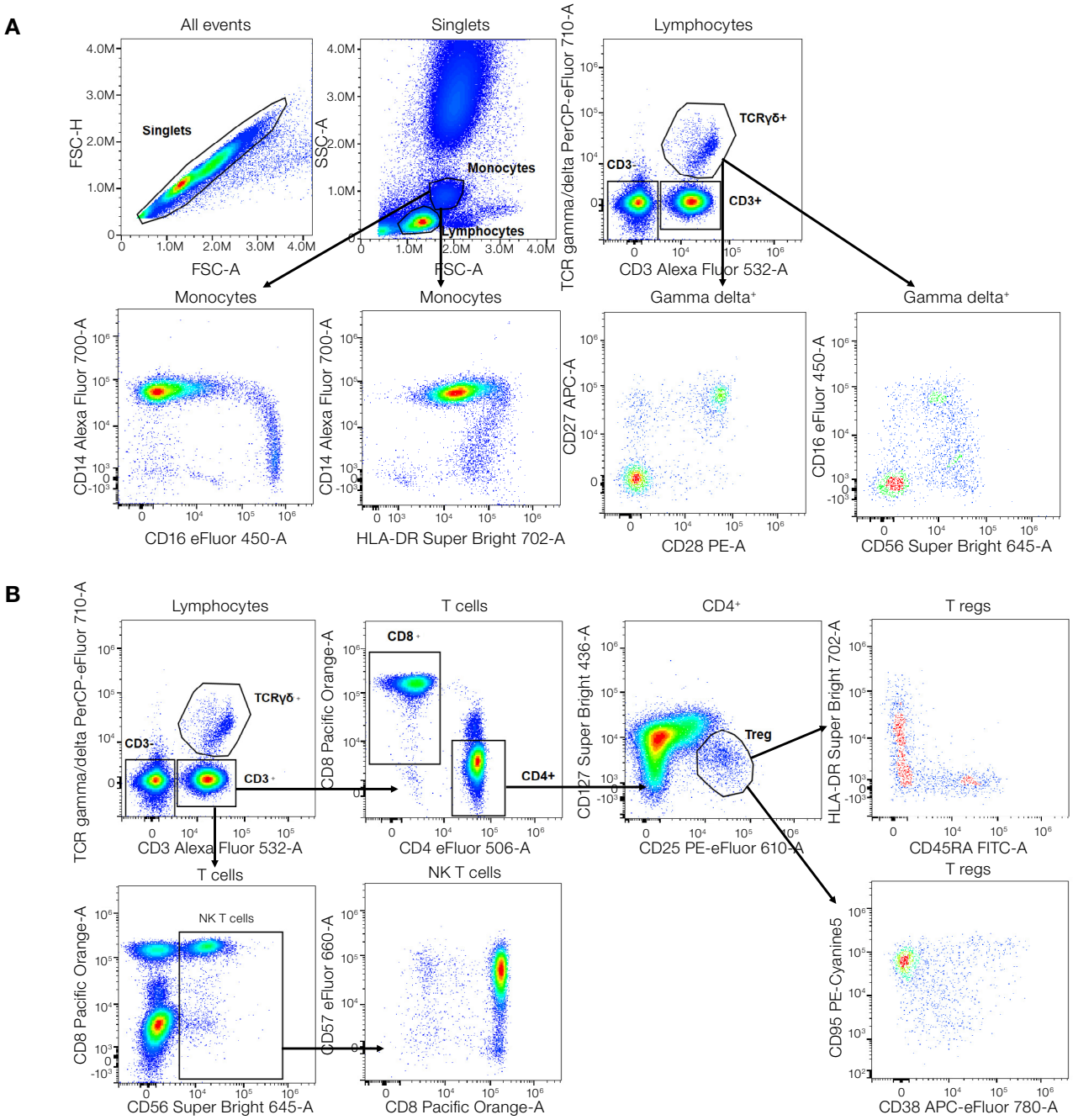

Figure 2. Example of a 20-color panel using Invitrogen fluorophore–labeled antibody conjugates to separate different monocyte and lymphocyte T cell populations. Human peripheral blood mononuclear cells (PBMCs) were isolated and stained with various immunological and sub-lineage markers. **(A)** Initial scatter gating separated monocyte populations to analyze their relative expression of common monocyte markers. Gamma delta T cell populations were also separated from T cell and non–T cell lymphocyte populations. **(B)** $CD3^+$ lymphocytes were further analyzed for cytotoxic CD8 and helper CD4 T cells. T regulatory cells were then isolated from $CD4^+$ cells, while natural killer (NK) T cells were identified from $CD3^+$ cells.

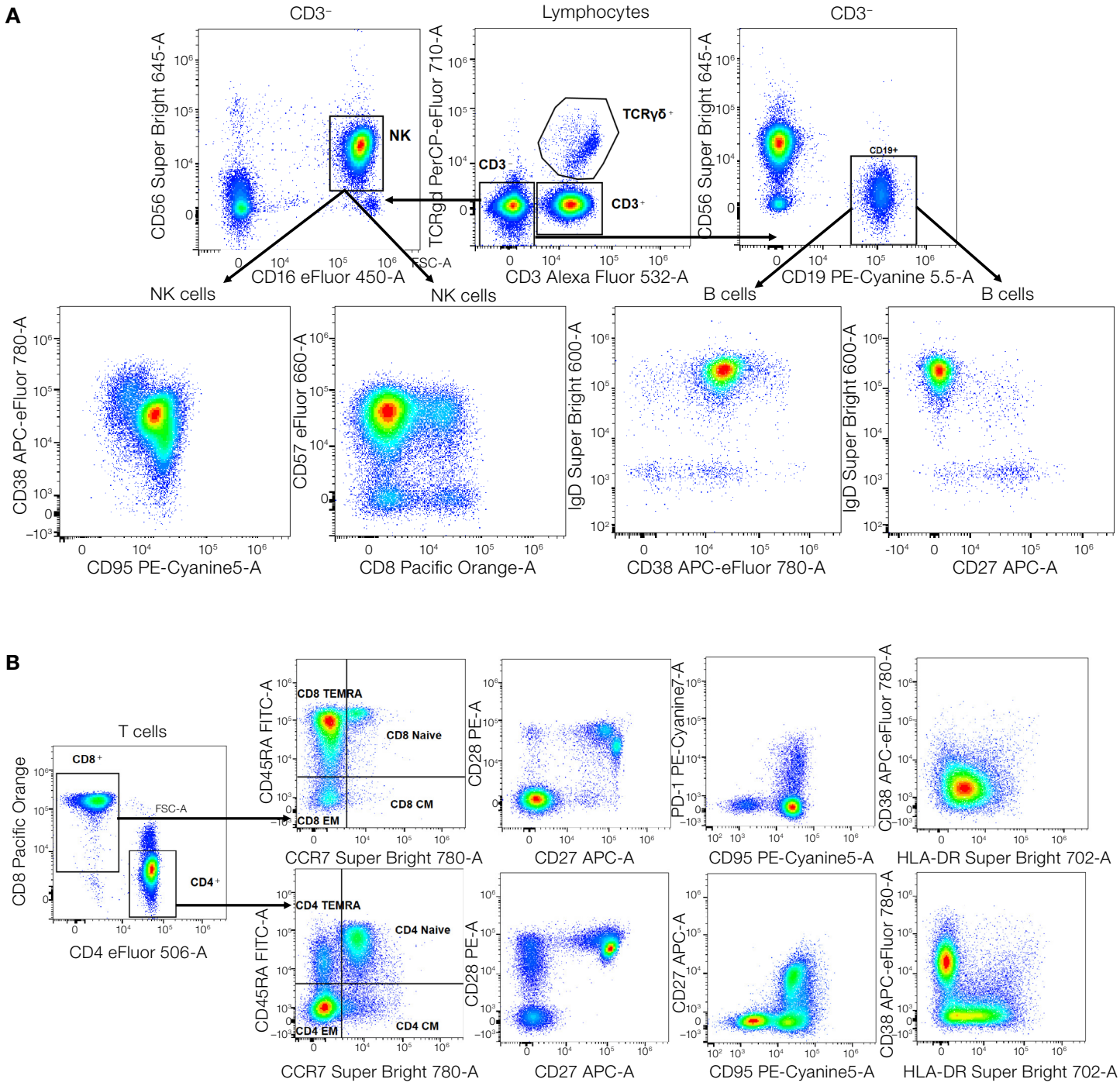

Figure 3. Example of a 20-color panel using Invitrogen fluorophore–labeled antibody conjugates to separate different T cell and B cell populations. Human peripheral blood mononuclear cells (PBMCs) were were isolated and stained with various immunological and sub-lineage markers. **(A)** CD3⁻ cells were separated into NK cells and B cells, using CD56 and CD19, respectively. **(B)** Further gating and analysis of CD4⁺ and CD8⁺ T cells using CCR7 and CD45 allowed for the identification of naive, effector memory, central memory, and re-expressing effector memory CD45RA T cell subsets. Note: Data shown on Figures 2 and 3 are obtained from the same experiment.

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A 20-color spread matrix was generated with Invitrogen antibodies to demonstrate their use on a 3-laser spectral flow cytometer* (Table 2). The spread matrix was used as a guide to help select the appropriate antibody conjugates that were used in the previous 20-color panel. Many previously incompatible fluorophores are now compatible, including Invitrogen™ Super Bright 436 with eFluor™ 450 dye, eFluor™ 506 dye with Pacific Orange, and APC with Alexa Fluor™ 647 dye.

Use function-based approaches to understand the biological significance of your spectral flow cytometry experiment. Invitrogen™ LIVE/DEAD™ Fixable Viability Stains, cell cycle reagents, apoptosis probes, and others, are all compatible with spectral flow cytometer systems. Figure 4 is a demonstration of the Invitrogen™ PrimeFlow™ RNA Assay and the ability of a spectral flow cytometer to separate true fluorescent signals from autofluorescence.

	Violet laser (405 nm)								Blue laser (488 nm)								Red laser (635 nm)			
	Super Bright 436	eFluor 450	eFluor 506	Pacific Orange	Super Bright 600	Super Bright 645	Super Bright 702	Brilliant Violet 785	FITC	Alexa Fluor 532	PE	PE-eFluor 610	PE-Cy5	PE-Cy5.5	PerCP-eFluor 710	PE-Cy7	APC	Alexa Fluor 647	Alexa Fluor 700	Alexa Fluor 780
Super Bright 436																				
eFluor 450																				
eFluor 506																				
Pacific Orange																				
Super Bright 600																				
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PE-Cy5																				
PE-Cy5.5																				
PerCP-eFluor 710																				
PE-Cy7																				
APC																				
Alexa Fluor 647																				
Alexa Fluor 700																				
APC-eFluor 780																				

Table 2. Staining spread matrix of 20 Invitrogen fluorophores that can be used simultaneously on a 3-laser spectral flow cytometer.* All fluorophores were compared using anti-CD4 antibody conjugates to demonstrate the level of spread among dyes. The fluorophore in each row impacts the resolution of the fluorophore in each column. Although all dyes in the matrix can be used together, the darker red shading means one fluorophore has increased spread into the other and needs closer attention during panel design and data interpretation.

PrimeFlow RNA Assay

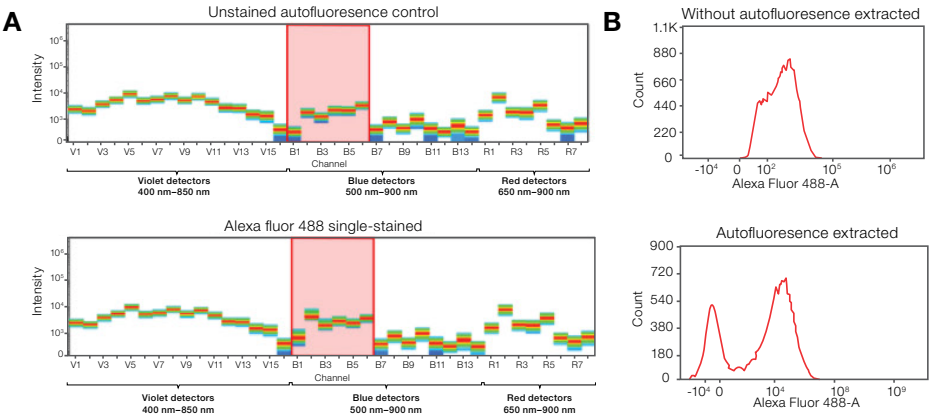

Figure 4. Example of autofluorescence extraction using the PrimeFlow RNA kit. mRNA from human U937 cells was labeled with PrimeFlow RNA Assay probes. **(A)** Cells were treated with PrimeFlow RNA detection reagent and were either unstained (top) or stained (bottom) before analysis on a 3-laser spectral flow analyzer.* **(B)** Unstained cells were mixed with stained cells, and analyzed before (left) and after (right) autofluorescence removal.

* All spectral flow cytometry data shown were generated by Cytek Biosciences on a Cytek™ Aurora™ spectral flow cyometer 3-laser system and analyzed using SpectroFlo™ software.

Select the right reagent at
thermofisher.com/spectralflowcytometry

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