Add markers with minimal impact to compensation using NovaFluor dyes

Narrow excitation spectra enable more fluorophores per panel

Invitrogen™ NovaFluor™ dyes are designed for greater data resolution with narrow emission spectra and minimal cross-laser excitation profiles. NovaFluor dyes have unique spectra, with decreased spillover into other dyes, resulting in higher resolution. Spectral overlap or spillover into multiple detectors may reduce the resolution of the fluorescence signal. NovaFluor dyes can aid in panel design because they have narrow laser excitation ranges (Figure 1) and emission profiles.

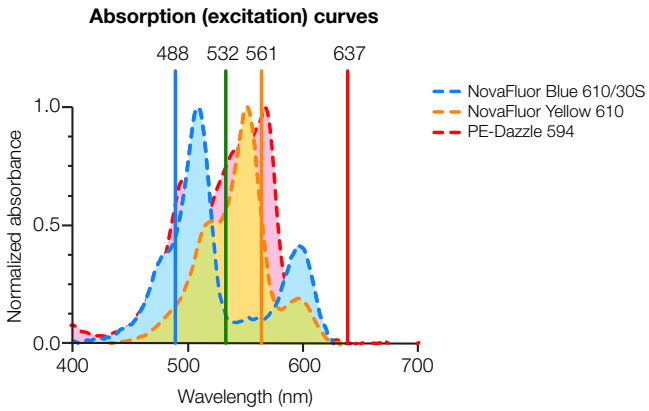

Figure 1. NovaFluor dyes have tighter excitation ranges than PE-Dazzle™ 594 dye. Normalized absorbance spectra of PE-Dazzle 594 (BioLegend), Invitrogen™ NovaFluor™ Blue 610/30S, and NovaFluor™ Yellow 610 dyes with blue (488 nm), green (532 nm), yellow (561 nm), and red (637 nm) laser lines overlaid. Excitation of PE-Dazzle 594 dye by the blue and yellow/green lasers leads to occupation of the detectors on both laser lines. Swapping PE-Dazzle dye with NovaFluor Blue 610/30S and NovaFluor Yellow 610 dyes will allow you to gain one additional marker.

Advantages of NovaFluor dyes

- Engineered to have narrow excitation and emission profiles to help gain detectors and fit additional markers
- Designed for less spillover and low spectral spread for higher resolution of populations and easier panel construction
- Available with different levels of brightness to reduce spread for high-expression antigens
- Built using Phiton™ technology (Phitonex)—a DNA scaffold labeled with small-molecule fluorophores—that lends stability, NovaFluor dyes retain fluorescence intensity and spectral signature at long-term 4°C storage after staining and fixation

The Invitrogen™ CellBlox™ Blocking Buffer is formulated to block nonspecific binding of NovaFluor dye labels to cells. These nonspecific interactions can result in higher background labeling. To minimize background labeling, CellBlox Blocking Buffer should be used in conjunction with a NovaFluor dye to label any cell type.

CellBlox Blocking Buffer is also recommended for use with cyanine-based dyes and tandem dyes to block nonspecific interactions with monocytes and macrophages, thereby minimizing background labeling.

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Increase panel size by replacing conventional tandem dyes with unique NovaFluor dyes

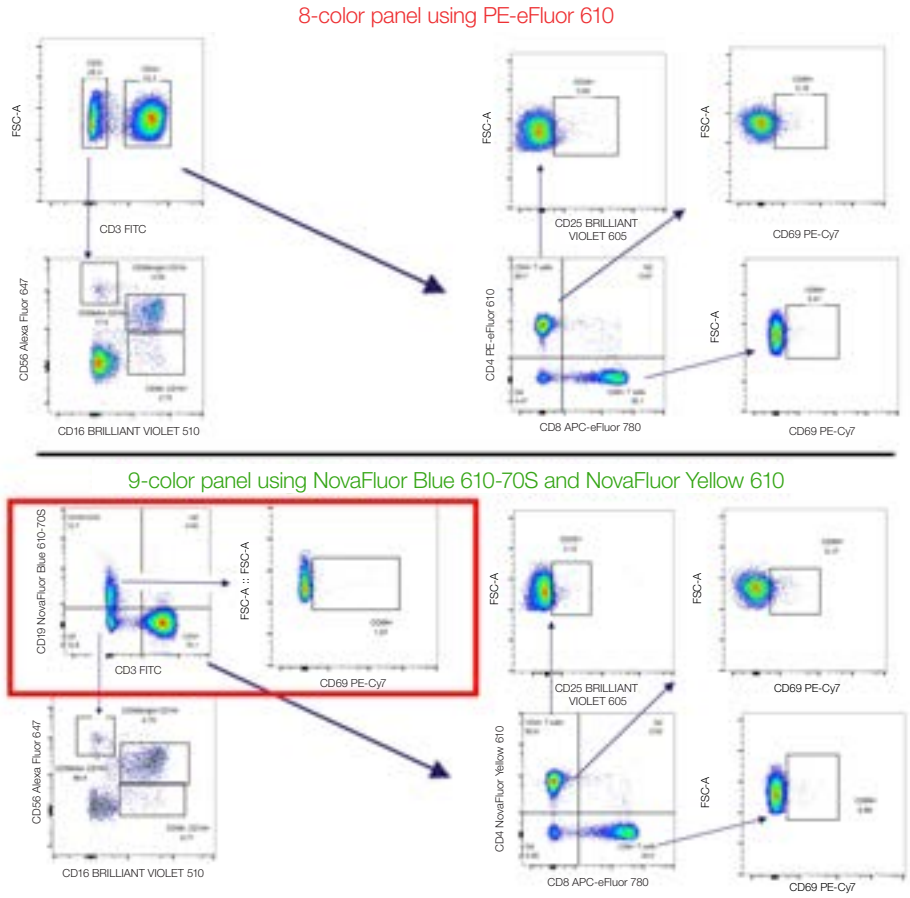

Figure 2. Replacing a conventional tandem dye with NovaFluor dyes allows incorporation of additional markers in the panel. Normal human peripheral blood mononuclear cells (1 x 10^6 cells/well) were stained in 1X PBS using 1 µL of Invitrogen™ LIVE/DEAD™ Fixable Violet stain (Cat. No. **50-113-8086**) for 30 minutes. Cells were washed and then blocked using CellBlox buffer (Cat. No. **B001T03F01**) and Invitrogen™ eBioscence™ Fc Receptor Binding Inhibitor Polyclonal Antibody (Cat. No. **50-138-03**) in Invitrogen™ eBioscience™ Flow Cytometry Staining Buffer (Cat. No. **50-112-9748**). Cells were then stained in two separate panels, the **top panel**: using anti-human CD4 Invitrogen™ eBioscience™ PE-eFluor™ 610 dye (5 µL, Cat. No. **50-112-9370**) and the **bottom panel**: replacing the PE-eFluor 610 dye with anti-human CD4 NovaFluor Yellow 610 dye (5 µL, Cat. No. **H001T03Y03**) and anti-human CD19 Invitrogen™ NovaFluor™ Blue 610-70S dye (5 µL, Cat. No. **H004T03B06**). Cells were stained for 30 minutes at 4°C, washed, and then fixed using 100 µL of Invitrogen™ eBioscience™ IC Fixation Buffer (Cat. No. **50-112-9058**) overnight. Cells were then washed with Flow Cytometry Staining Buffer and data were collected on an Invitrogen™ Attune™ CytPix™ Flow Cytometer (4-laser configuration) from 30,000 cells from the lymphocyte gate. Collected data were compensated and later analyzed using FlowJo™ software (BD Biosciences™). The red box in the table and figure shows panel expansion is now possible for an additional marker by using NovaFluor dyes.

Setup with conventional tandem dyes (8-color panel)*	Fluorophore dye	Marker	Setup with NovaFluor dyes (9-color panel)*	Fluorophore dye	Marker
V1	LIVE/DEAD Violet	Viability	V1	LIVE/DEAD Violet	Viability
V2	BRILLIANT VIOLET 510	CD16	V2	BRILLIANT VIOLET 510	CD16
V3	BRILLIANT VIOLET 605	CD25	V3	BRILLIANT VIOLET 605	CD25
V4			V4		
B1	FITC	CD3	B1	FITC	CD3
B2			B2	NovaFluor Blue 610-70S	CD19
B3			B3		
Y1			Y1		
Y2	PE-eFluor 610	CD4	Y2	NovaFluor Yellow 610	CD4
Y3			Y3		
Y4	PE-Cy7	CD69	Y4	PE-Cy7	CD69
R1	Alexa Fluor 647	CD56	R1	Alexa Fluor 647	CD56
R2			R2		
R3	APC-eFluor 780	CD8	R3	APC-eFluor 780	CD8

* V = violet, B = blue, Y = yellow, and R = red

Visit **thermofisher.com/flowpanel** for no-charge panel design service with help from technical support specialists.
Visit **thermofisher.com/novafluor** to learn more about NovaFluor dyes.

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