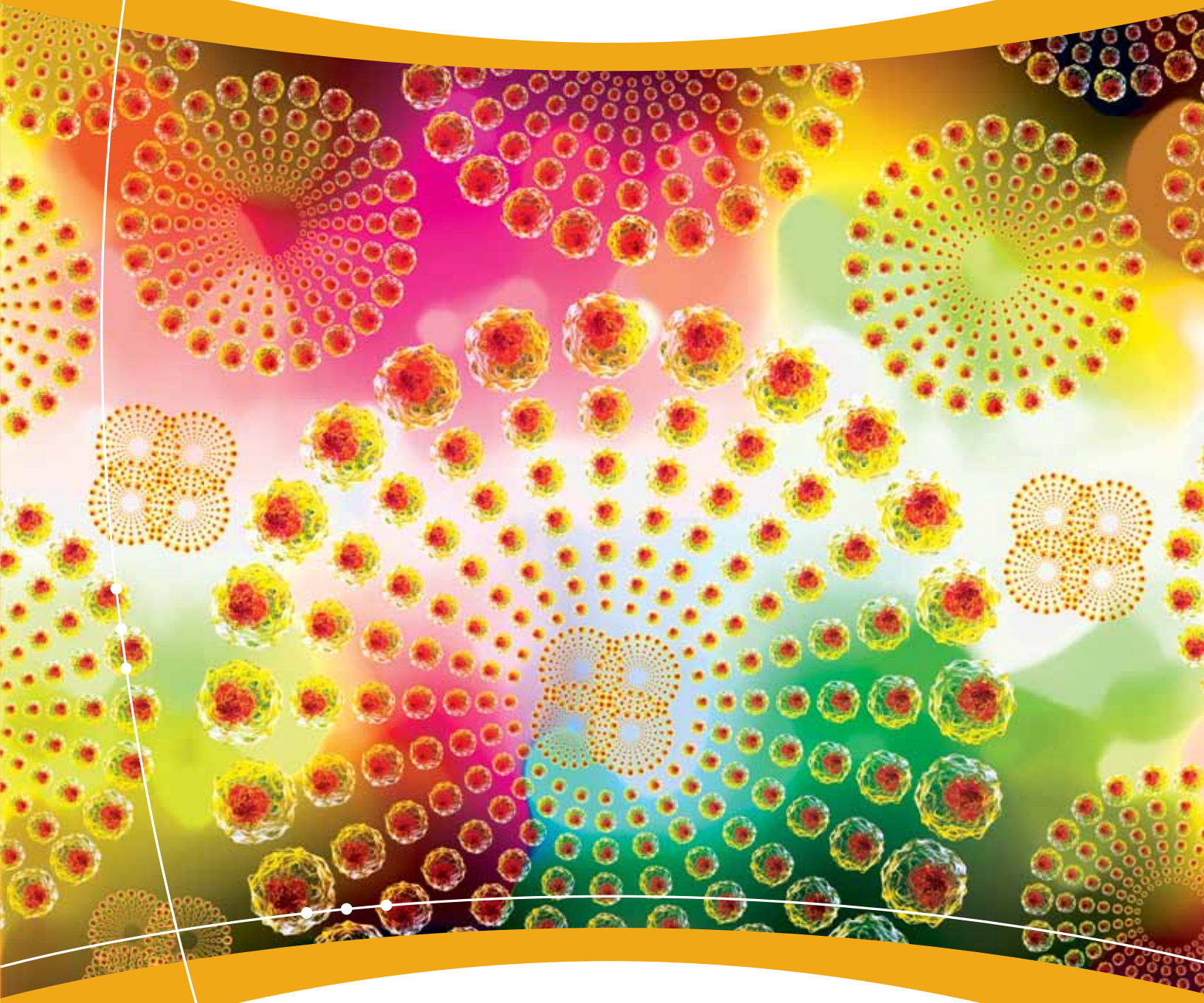
eBioscience®
high performance immunoassays

Multicolor Flow Cytometry

Technical Resource Guide



eBioscience®
high performance immunoassays

eBioscience is committed to developing and manufacturing high-quality, innovative reagents in an ISO certified facility. As a provider of more than 10,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.

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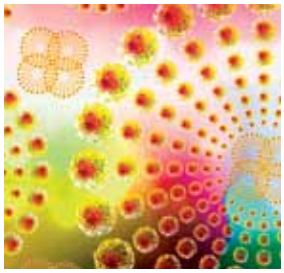


Multicolor Flow Cytometry

Technical Resource Guide

Flow cytometry is an indispensable technique for deciphering complex cellular processes and interactions in a variety of systems that model normal and disease states. The development of cytometers equipped with 3-5 lasers and capable of detecting >15 fluorescent parameters has made flow cytometry a fundamental tool for the life scientist. Realizing the power of multicolor flow cytometry requires high performance fluorophores paired with appropriate specificities to ensure consistent and reliable data.

As the industry leader in reagent offerings for multicolor flow cytometry, our goal is to provide the most comprehensive and innovative portfolio of antibody conjugates for multicolor applications. eFluor® is the next generation of organic and nanocrystal-based fluorophores developed and optimized for multiparameter flow cytometry. The eFluor® brand consists of the following product lines:

eFluor® NANOCRYSTALS (NC)

- Direct conjugates for flow cytometry
- Narrow emission spectra and excellent photostability
- Expands the utility of violet laser flow cytometry

eFluor® ORGANIC DYES

- Direct conjugates for flow cytometry
- Robust performance for all work flow scenarios

eFluor® FUNCTIONAL DYES

- Small molecule organic dyes
- Viability dyes for intracellular staining protocols
- Proliferation dyes for tracking cell division
- Fluorescent dyes to monitor intracellular free calcium

Our research and development team continues to improve and expand our fluorophore portfolio to provide you with reagents that give optimal resolution of targets for multicolor flow cytometry applications.

Quality First. Value Always.

A commitment to quality and value is the philosophy that guides our everyday operations and represents our obligation to customers who rely on our reagents and support products for flow cytometry. At eBioscience, we manufacture our products to meet stringent performance specifications so you can feel confident that our reagents will perform consistently over time. Our scientists are committed to keeping pace with the dynamic changes in the life sciences by developing relevant, high quality reagents at an accelerated pace. We pride ourselves in being first to market with many critical reagents and will continue to develop the finest reagents to support multicolor flow cytometry. All of our products are supported by a highly respected Technical Support team that is staffed by experienced scientists to ensure that eBioscience customers get the maximum return on their reagent investment.

Using This Resource Guide

For each laser (Violet, Blue, Red) we highlight the spectral properties of the fluorophores offered by eBioscience for use with that laser, as well as information concerning compensation and related issues for each.

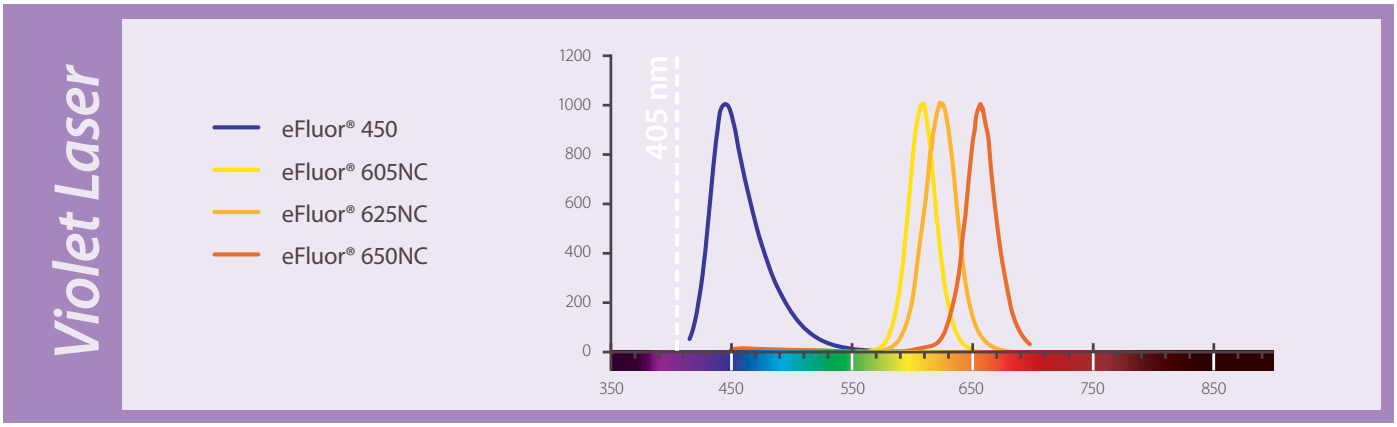
A Note Regarding Data

All compensation data presented in this brochure are assessed on a single stain of a sample. All eFluor® NC were stained according to eBioscience recommendations using eFluor® NC staining buffer. Fixation studies were performed by resuspending stained cells in 100 µl of staining buffer and adding 100 µl of 4% Paraformaldehyde (PF) to achieve a final concentration of approximately 2% PF. Except where indicated, cells were left in fixative for 48 hours at 4^0C, protected from light prior to acquisition. All data were acquired on a BD LSRII.

Violet Laser (405, 407 nm)

The violet laser is quickly becoming a standard component of multi-laser flow cytometers and is often used for common phenotyping markers in multicolor staining panels. eBioscience offers four eFluor® reagents: eFluor® 450, eFluor® 605NC, eFluor® 625NC and eFluor® 650NC, for use with the violet laser. These reagents work together to provide maximum resolution of antigenic determinants when used in a multicolor panel. This ease-of-use feature is accomplished by narrow emission peaks that result in little spectral overlap and, therefore, require minimal intra-laser compensation. In addition to violet laser excitation, the eFluor® nanocrystal (NC) reagents can also be excited with a UV (355 nm) laser making them versatile reagents for multicolor flow cytometry and compatible with various instrument configurations. For design of staining panels containing three violet laser reagents, we recommend using eFluor® 450, eFluor® 605NC and eFluor® 650NC as these minimize issues of spectral overlap of emitted light. The optimal performance of eFluor® NC reagents is achieved by using eFluor® NC Flow Cytometry Staining Buffer for all antibody incubations which is suitable for use with staining panels containing both conventional fluorophores and nanocrystal reagents.

Emission Spectra for eBioscience Violet Laser Fluorophores



Violet Laser Dyes

eFluor® 450: An organic dye that emits at 450 nm. This small molecule fluorescent dye has similar spectral properties to Pacific Blue® and was developed by eBioscience to have equal or better fluorescence intensity when compared to Pacific Blue® in multicolor applications. eFluor® 450 is a competitive replacement for Pacific Blue® or Horizon™ V450 in any multicolor staining panel.

eFluor® 605NC: A nanocrystal-based reagent that features a very narrow emission spectra to maximize signal to noise and minimize compensation. eFluor® 605NC is a brighter alternative to Pacific Orange®. A direct comparison of anti-mouse B220 (clone RA3-6B2) conjugated to eFluor® 605NC or Pacific Orange® revealed an approximately two-fold

improvement in the calculated stain index for eFluor® 605NC over Pacific Orange®. The individual signals compared were collected through optimal bandpass filters as specified by the manufacturer. For instruments not equipped with a violet laser, the eFluor® 605NC is adequately excited by the 488 nm blue laser and can be used as an alternative for PE-Texas Red®. For instruments equipped with blue and violet lasers and configured for detection of PE-Texas Red®, it is not recommended eFluor® 605NC be used simultaneously with PE-Texas Red®.

eFluor® 625NC: A nanocrystal-based reagent that features a very narrow emission spectra to maximize signal to noise and minimize compensation. Because its emission profile is shifted only 20 nm from eFluor® 605NC, eFluor® 625NC reagents should not be used simultaneously with eFluor® 605NC. eFluor® 625NC reagents do work well in combination with eFluor® 450; however, for those instruments capable of detecting >2 fluorescent parameters from the violet laser, we recommend use of eFluor® 450, eFluor® 605NC and eFluor® 650NC for optimal results.

eFluor® 650NC: Our brightest nanocrystal-based reagent for flow cytometry is an easy to use reagent for instruments configured to measure three fluorescent parameters from the violet laser.

Compensation Considerations For Violet Laser Dyes

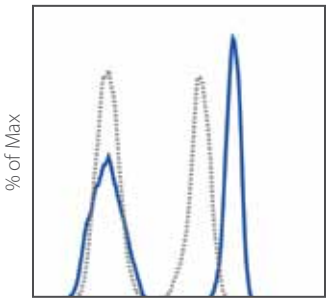
Compensation is always specific for the particular combination of specificities and fluorophores in a multicolor staining panel and, therefore, needs to be calculated for each experiment. The compensation matrix in the table below shows representative data for the percent of compensation required from the indicated detectors for human PBMC samples stained with our violet-excited eFluor® 450, eFluor® 605NC or eFluor® 650NC dyes conjugated to anti-CD4. These reagents for the violet laser perform very well together and exhibit minimal spectral overlap with each other. However, because the eFluor® 650NC is excited to some extent by the red laser and its peak emission is 650 nm, it does require some compensation from the APC detector.

Compensation Matrix for Violet Laser (405, 407 nm) Excited Fluorophores

(Actual Stain)	eFluor® 450	eFluor® 605NC	eFluor® 650NC	FITC	PE	PerCP-eFluor® 710	PE-Cy7	APC	Alexa Fluor® 700	APC-eFluor® 780
eFluor® 450	--	1.23	0.02	0.02	0.00	0.04	0.05	0.01	0.03	0.05
eFluor® 605NC	0.23	--	1.65	0.00	0.73	0.00	0.01	0.00	0.00	0.02
eFluor® 650NC	0.05	3.20	--	0.00	0.00	10.77	0.04	34.63	0.81	0.01

Fixation Considerations For Violet Laser Dyes

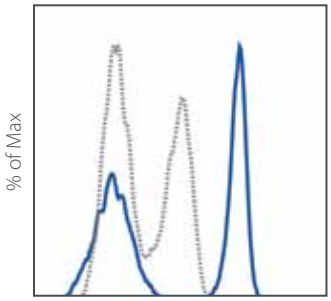
The ability of fluorophore conjugated reagents to retain fluorescence performance after fixation is a critical parameter necessary to accommodate all work flow scenarios. Some generalizations regarding fluorophore performance after fixation can be made, but clone-specific performance should be determined empirically. In the data shown below, human PBMCs were stained with eFluor® 450, eFluor® 605NC or eFluor® 650NC conjugated to anti-CD4 or anti-CD3 prior to fixation. The blue histogram represents staining of unfixed cells and the grey dotted histogram represents staining of fixed cells.



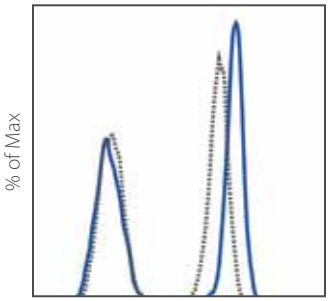
CD4 (SK3) eFluor® 450

eFluor® 450

Under conditions where the cell sample was left in 2% Paraformaldehyde (PF) for 48 hours, there is an approximately 60% reduction in MFI following fixation. We recommend a shorter exposure to fixative, and have seen minimal loss of fluorescence when cells are exposed to fixative for ≤ 1 hour.



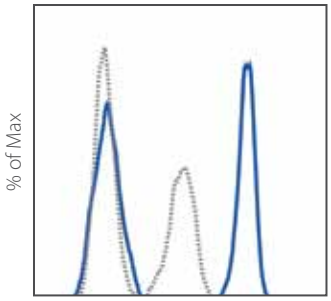
CD4 (OKT4) eFluor® 605NC



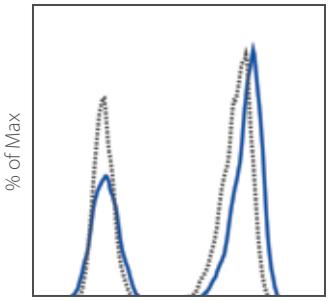
CD4 (OKT4) eFluor® 605NC

eFluor® 605NC and eFluor® 650NC

The composition of nanocrystals makes these reagents much more sensitive to fixation conditions than conventional organic dyes or fluorescent proteins. This can be observed in the data shown here for both the 605NC and 650NC (data boxes on left for each pair) when exposed to 2% paraformaldehyde for 48 hours. We have found that limiting the time of fixation to ≤ 30 minutes results in minimal loss of fluorescence (data boxes on right for each pair).



CD4 (RPA-T4) eFluor® 650NC



CD3 (OKT3) eFluor® 650NC

Viability Detection Reagents For The Violet Laser

Excluding dead cells from analysis is highly recommended for all flow cytometry experiments to minimize the potential artifacts introduced by antibody conjugates binding nonspecifically to dead cells. eBioscience offers our Fixable Viability Dye eFluor® 450 for the violet laser, which is ideal for use with intracellular staining protocols.

Fixable Viability Dye eFluor® 450: This dye preferentially and permanently labels dead cells, thereby allowing them to be excluded from analysis. Fixable Viability Dyes are amine-reactive fluorescent reagents that bind free amine groups on proteins. They are not membrane permeable and, therefore, only minimally label live cells. However, dead and dying cells with compromised membranes allow access of the dye to the interior of the cell resulting in a very bright fluorescence of the dead cell population. These dyes are fixable and are retained in cells following the fixation and permeabilization steps required in intracellular staining protocols.

Calcein Violet AM: This dye can be used to identify live cells by flow cytometry. Upon entry into the cell, intracellular esterases cleave the acetoxymethyl (AM) group to yield a membrane-impermeable calcein fluorescent dye. Calcein Violet AM is not retained in dead or dying cells with compromised cell membranes and, therefore, is not suitable for intracellular staining protocols.

Note: Products for viability detection on the violet laser cannot be used in combination with eFluor® 450, Pacific Blue® or any other violet laser excited fluorophore that emits light in the 450 nm range.

Ordering Information

Product	Catalog Number	Application Notes	Excitation Laser (nm)	Peak Emission (nm)
Fixable Viability Dye eFluor® 450	65-0863	Permanently labels dead cells Ideal for intracellular staining protocols	405, 407	450
Calcein Violet AM	65-0854	Identifies live cells by flow cytometry Measures intracelllular esterase activity	405, 407	450

Filter Recommendations For eBioscience Fluorophores For The Violet Laser

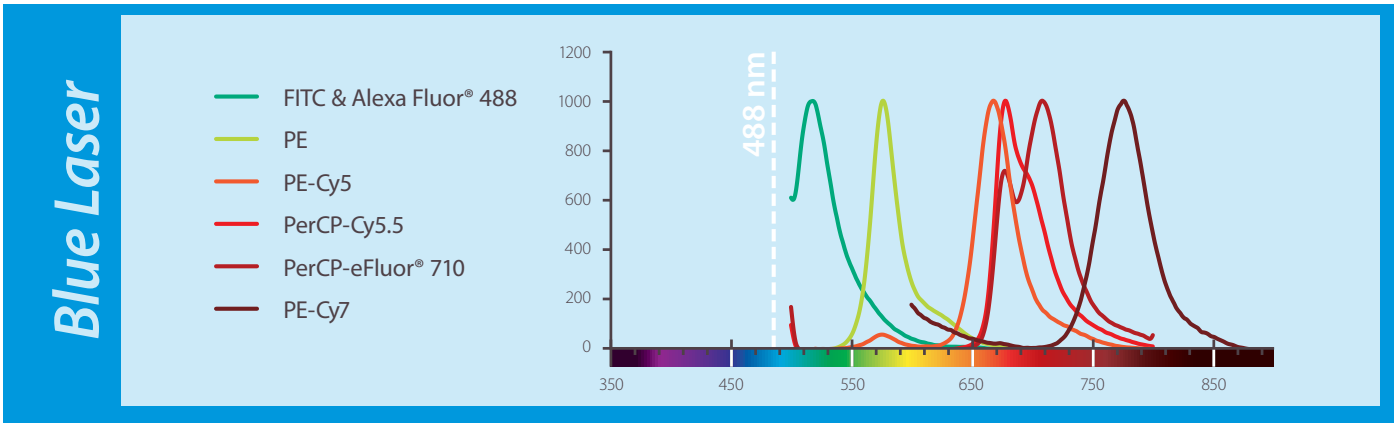
Violet Laser	Excitation Laser (nm)	Peak Emission (nm)	Dichroic Mirror	Band Pass Filter
eFluor® 450	405, 407	450	--	440/40, 450/50
eFluor® 605NC	355, 405, 407	605	595 LP	605/40, 605/50, 605/20, 610/20
eFluor® 625NC	355, 405, 407	625	595 LP	605/50, 625/20
eFluor® 650NC	355, 405, 407	650	630 LP	655/20, 660/40

New products are launched regularly. **Discover more at www.eBioscience.com.**

Blue Laser (488 nm)

Virtually all flow cytometers are equipped with a blue laser (488 nm) and, in general, at least 3 fluorescent parameters can be measured using this excitation wavelength. Historically, these three parameters were designated as FL1 (FITC), FL2 (PE), and FL3. More sophisticated instruments are designed to measure 4 or 5 fluorescent parameters simultaneously from the 488 nm laser.

Emission Spectra for eBioscience Blue Laser Fluorophores



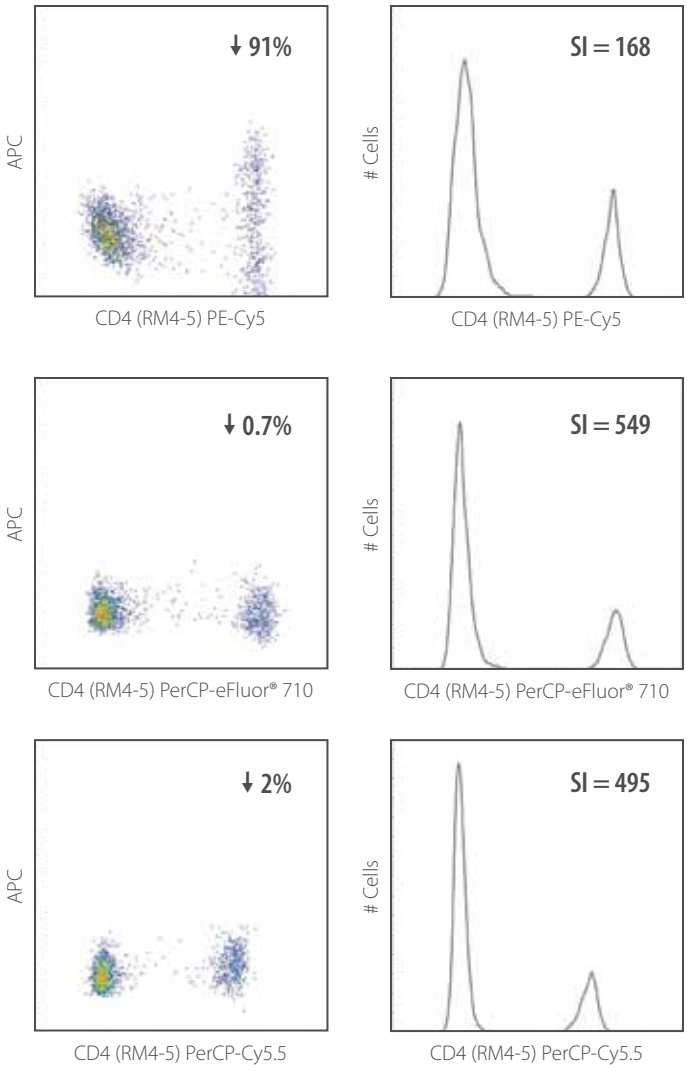
Blue Laser Dyes

FITC (Fluorescein Isothiocyanate) and Alexa Fluor® 488: These are small molecule fluorescent dyes that emit light of approximately 520 nm; they cannot be used in the same sample. Historically the detector for these fluorophores has been designated as FL1.

PE (Phycoerythrin): A 240 kD fluorescent phycobiliprotein that emits light of approximately 575 nm. PE is considered to be one of the brightest fluorophores with a high stain index and, therefore, great separation of positive and negative events. PE is a good choice when the expression level of an antigen of interest is not known or is known to be expressed at low levels. Historically the detector for PE has been designated as FL2.

PE-Cy5, PerCP-Cy5.5 and PerCP-eFluor® 710: These are tandem dyes that are composed of a protein donor molecule and a small organic acceptor molecule. PE-Cy5 uses PE as its donor while the PerCP (Peridinin chlorophyll protein) tandems are constructed using the 35 kD fluorescent protein from the dinoflagellate, *glenodinium*. Although PE-Cy5 provides a robust signal to noise ratio, it requires a significant amount of cross-beam compensation out of the APC detector. A better alternative for multicolor staining panels is a PerCP-tandem dye such as, PerCP-Cy5.5 and PerCP-eFluor® 710. PerCP-eFluor® 710 is the newest member of the eFluor® Organic Dyes product line and is two to threefold brighter than PerCP-Cy5.5. PerCP-eFluor® 710 is comparable to PE-Cy5 in

fluorescence intensity and is free of the cross-beam compensation issues associated with PE-Cy5. PerCP-tandem dyes from eBioscience are both brighter and more photostable than the PerCP molecule alone. While we continue to offer PE-Cy5 conjugates, we recommend their use only with instruments equipped with a yellow/green (532-561 nm) laser.



Comparison of FL3 Choices

For comparison of performance, this example shows mouse splenocytes stained with the anti-CD4 (clone RM4-5) conjugated to PE-Cy5 (top panels), PerCP-eFluor® 710 (middle panels), or PerCP-Cy5.5 (bottom panels). The compensation required to remove the PE-Cy5 signal from the APC detector is shown in the dot plots for each conjugate. The compensation requirement for the PE-Cy5 tandem out of the APC detector can range from 50-90% depending on the specificity of the antibody and the cell type being analyzed. In the staining results shown here, the extreme compensation required for PE-Cy5 from the APC detector results in a large spread of the CD4 positive population that does not occur with either of the PerCP-tandem conjugates. Moreover, calculating the stain index for clone RM4-5 in each format to compare the resolution obtained for each fluorophore, both of the PerCP tandem dyes provide better resolution of the positive population from the negative than that obtained with PE-Cy5. Finally, comparing the stain index values for both PerCP tandem dyes, PerCP-eFluor® 710 from eBioscience clearly provides the greatest resolution of the target cells.

PE-Cy7: PE-Cy7 is a tandem dye that has been optimized for FRET efficiency and minimal spillover into the PE detector. Upon excitation, the majority of the PE emission excites the Cy7 acceptor dye, which has a peak emission of approximately 785 nm. Prolonged exposure of PE-Cy7 to light results in some reduction in the FRET efficiency of the tandem and, therefore, an increase in the amount of compensation required out of the PE detector.

Compensation Considerations For Blue Laser Dyes

Compensation is always specific for the particular combination of specificities and fluorophores in a multicolor staining panel and, therefore, needs to be calculated for each experiment. To provide a general guideline for where to expect compensation when using blue laser-excited fluorophores from eBioscience in multicolor staining panels, human PBMC samples were stained with anti-CD4 conjugated to the indicated fluorophores. The percent of compensation required for each fluorophore is shown for the indicated detector. With the exception of PE-Cy5, fluorophores that are optimally excited with the blue laser require very little cross-beam compensation.

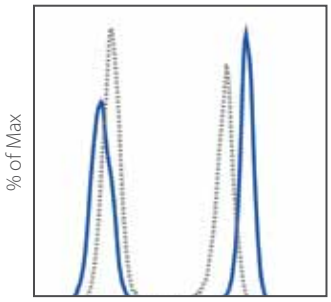
Compensation Matrix for Blue Laser (488 nm) Excited Fluorophores

(Actual Stain)	eFluor® 450	eFluor® 605NC	eFluor® 650NC	FITC	PE	PerCP-eFluor® 710	PE-Cy7	APC	Alexa Fluor® 700	APC-eFluor® 780
FITC	0.00	0.81	0	--	14.69	0.73	0.19	0.00	0	0.01
Alexa Fluor® 488	0.00	0.81	0	--	12.49	0.73	0.19	0.00	0	0.01
PE	0.00	7.64	0.95	1.27	--	7.17	0.98	0.01	0.00	0.00
PE-Cy5	0.00	0.09	4.63	0.00	1.80	--	21.57	68.00	40.04	6.05
PerCP-Cy5.5	0.00	0.09	2.66	0.00	0.00	--	23.27	0.65	16.24	2.75
PerCP-eFluor® 710	0.00	0.09	0.65	0.00	0.00	--	26.17	0.36	13.54	2.75
PE-Cy7	0.00	0.22	0.00	0.15	2.43	1.69	--	0.01	0.06	3.31

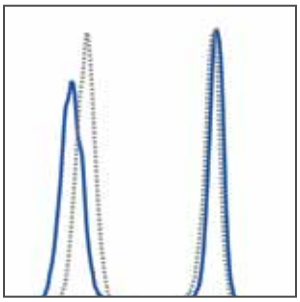
Fixation Considerations For Blue Laser Dyes

The data shown on page 10 represents an assessment of fluorophore performance after fixation. The ability of fluorophore conjugated reagents to retain fluorescence performance after fixation is a critical parameter necessary to accommodate all work flow scenarios. Some generalizations regarding fluorophore performance after fixation can be made, but clone specific performance should be determined empirically. In the examples shown on page 10, human PBMCs were stained with anti-CD4 reagents conjugated to our fluorophores for the blue laser prior to fixation. The blue histogram represents staining of unfixed cells and the grey dotted histogram represents staining of fixed cells.

Fixation Considerations For Blue Laser Dyes (continued)



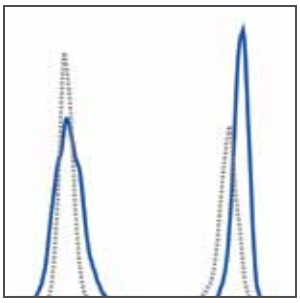
CD4 (SK3) FITC



CD4 (OKT4) Alexa Fluor® 488

FITC and Alexa Fluor® 488

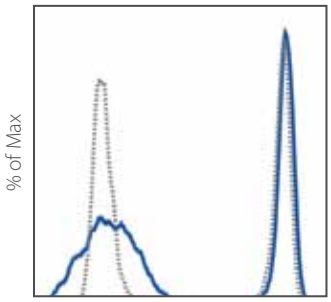
Both of these small molecule fluorescent dyes are amenable to fixation and although FITC generally shows some decrease in fluorescence intensity following fixation, in most cases the resolution of the positive population is unaffected.



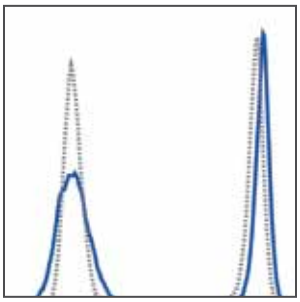
CD4 (SK3) PE

PE

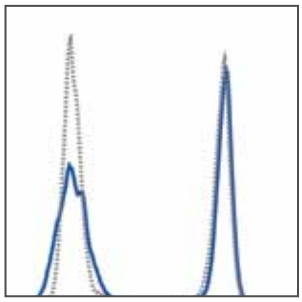
Fixation of PE conjugates will generally result in a 30-40% decrease in MFI. However, because the stain index of PE is so high and produces superb resolution of positive and negative populations, this decrease in MFI after fixation has little impact on interpretation of data obtained from cells stained with PE conjugated reagents.



CD4 (RPA-T4) PE-Cy5



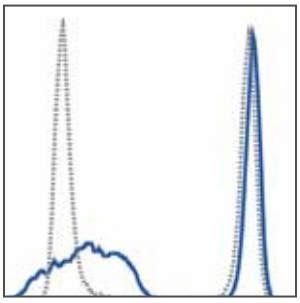
CD4 (SK3) PerCP-eFluor® 710



CD4 (RPA-T4) PerCP-Cy5.5

PE-Cy5, PerCP-eFluor® 710, and PerCP-Cy5.5

All three tandem dyes perform well when subjected to fixation with virtually no loss of fluorescent signal.



CD4 (RPA-T4) PE-Cy7

PE-Cy7

eBioscience PE-Cy7 has been optimized for performance and has virtually no loss of fluorescent signal when subjected to fixation. Additionally, fixation of this tandem dye does not increase the amount of compensation required from the PE detector.

Viability Detection Reagents For The Blue Laser

Dead and dying cells in a sample can result in false staining during flow cytometry experiments and confound interpretation of results. Excluding dead cells from analysis is highly recommended for all flow cytometry protocols to minimize the potential artifacts introduced by antibody conjugates binding nonspecifically to dead cells. eBioscience offers a variety of products for the blue laser that can be used to exclude dead and dying cells from analysis.

Calcein AM: Calcein AM is a membrane-permeable dye that can be used to identify live cells by flow cytometry. Upon entry into the cell, intracellular esterases cleave the acetoxymethyl (AM) group to yield a membrane-impermeable calcein fluorescent dye. This dye is not retained in cells with compromised plasma membranes and, therefore, is not retained in cells whose membranes have been permeabilized for intracellular staining with fluorescent antibody conjugates.

DNA Binding Dyes: Propodium Iodide and 7-AAD are membrane-impermeable fluorescent dyes that bind nucleic acid. They gain access to dead cells with compromised cell membranes and are excluded from cells with intact plasma membranes. These dyes are not retained in cells after the fixation and permeabilization steps required for intracellular (IC) staining and are not suitable in IC protocols.

Ordering Information

Product	Catalog Number	Application Notes	Excitation Laser (nm)	Peak Emission (nm)
Calcein AM	65-0853	Identifies live cells by flow cytometry Measures intracelllular esterase activity	488	515
Propidium Iodide	00-6990	Identifies dead cells by flow cytometry Binds double stranded DNA and RNA	488	617
7-AAD	00-6993	Identifies dead cells by flow cytometry Binds double stranded DNA	488	647

Filter Recommendations For eBioscience Fluorophores For The Blue Laser

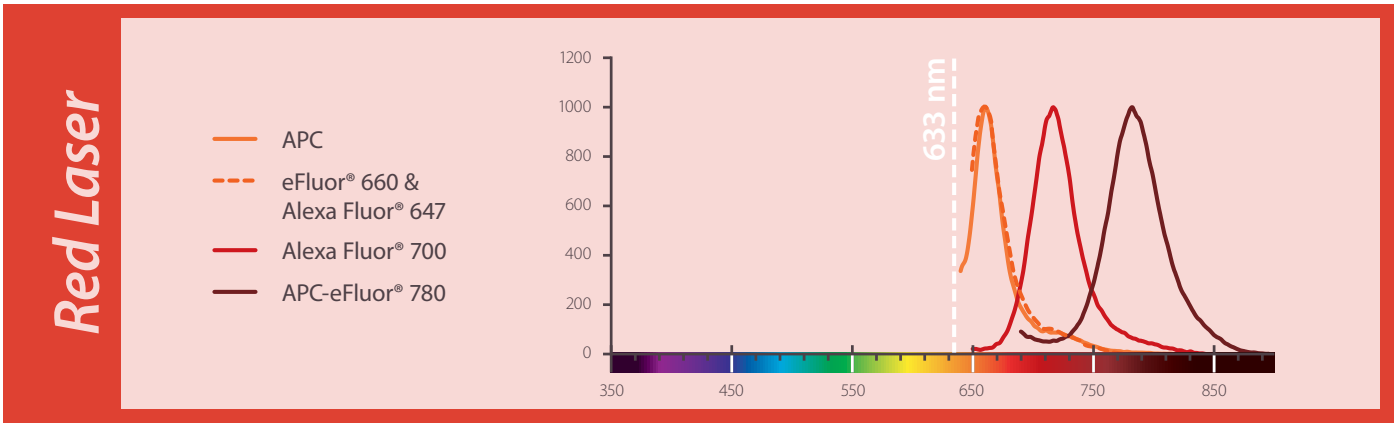
Blue Laser	Excitation Laser (nm)	Peak Emission (nm)	Dichroic Mirror	Band Pass Filter
FITC	488	518	---	530/30
Alexa Fluor® 488	488	519	---	530/30
PE	488	575	550 LP	575/26, 585/40
PE-Cy5	488	667	635 LP	670/14, 695/40
PerCP-Cy5.5	488	695	685 LP	695/40, 710/40
PerCP-eFluor® 710	488	710	685 LP	695/40, 710/40
PE-Cy7	488	785	735 LP	780/60, 780/40

New products are launched regularly. **Discover more at www.eBioscience.com.**

3 Red Laser (633 nm)

In addition to the blue (488 nm) laser, most instruments will also be configured with a red (633 nm) laser. The red laser is used for detection of APC and, on more advanced instruments, one or two additional fluorophores. Fluorophores excited by the 633 laser require little cross-beam compensation, but do require significant amounts of intra-laser compensation when used simultaneously.

Emission Spectra for eBioscience Red Laser Fluorophores



Red Laser Dyes

APC (Allophycocyanin), eFluor® 660 and Alexa Fluor® 647: APC is a fluorescent protein, while eFluor® 660 and Alexa Fluor® 647 are small organic molecules. All of these fluorescent molecules emit light in the 660-670 nm range and allow superb resolution of positive and negative populations. When eFluor® 660 or Alexa Fluor® 647 are used together with Alexa Fluor® 700 and APC-eFluor® 780, they require more compensation out of those detectors than APC. APC is considered to be one of the brightest fluorescent molecules available for flow cytometry and offers superb resolution of dim to medium expressed targets. APC is also a good choice when the expression level of an antigen of interest is not known.

Alexa Fluor® 700: A small molecule fluorescent dye that is useful in multicolor panels for staining antigens that are expressed at high levels. Alexa Fluor® 700 emits light of approximately 720 nm.

APC-eFluor® 780: A tandem dye composed of the protein donor molecule, Allophycocyanin, and a small organic molecule acceptor dye that has similar spectral properties to APC-Alexa Fluor® 750, APC-H7, and APC-Cy7. It is often the fluorophore of choice for the second red excited detector when the instrument is configured to measure two fluorescent parameters using the red laser.

Compensation Considerations For Red Laser Dyes

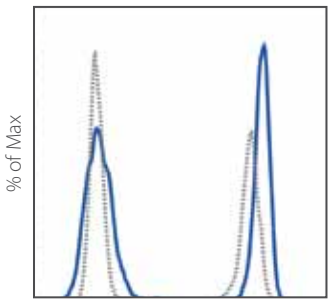
Compensation is always specific for the particular combination of specificities and fluorophores in a multicolor staining panel and, therefore, needs to be calculated for each experiment. The eBioscience fluorophores for use with the red laser require minimal cross beam compensation. The exception is Alexa Fluor® 700 which will require some compensation out of the detector for the PerCP tandems. However, when used simultaneously in a multicolor staining panel, significant compensation will be required between the red laser detectors. To provide a general guideline for where to expect compensation between detectors for red laser excited fluorophores, human PBMC samples were stained with eBioscience anti-CD4 conjugated to the indicated fluorophores. The percent of compensation required for each fluorophore is shown for the indicated detector.

Compensation Matrix for Red Laser (633-635 nm) Excited Fluorophores

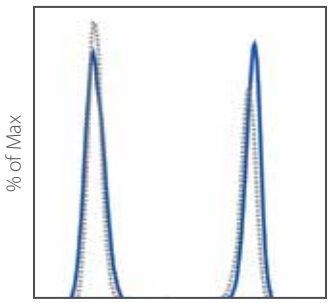
(Actual Stain)	eFluor® 450	eFluor® 605NC	eFluor® 650NC	FITC	PE	PerCP-eFluor® 710	PE-Cy7	APC	Alexa Fluor® 700	APC-eFluor® 780
APC	0.00	0.05	4.20	0.00	0.00	1.04	0.23	--	15.14	5.66
Alexa Fluor® 647	0.02	0.03	0.50	0.00	0.00	1.03	0.19	--	27.14	8.47
Alexa Fluor® 700	0.00	0.00	0.00	0.52	0.00	15.39	3.59	3.49	--	49.03
APC-eFluor® 780	0.00	0.00	0.67	0.00	0.00	0.49	4.21	20.63	3.97	--

Fixation Considerations For Red Laser Dyes

The following data are presented as an example of fluorophore performance following fixation with paraformaldehyde. The ability of fluorophore conjugated reagents to retain fluorescence performance after fixation is necessary to provide flexibility for a variety of work flow scenarios. Some generalizations regarding fluorophore performance after fixation can be made, but clone-specific performance should be determined empirically. In the examples shown here, human PBMCs were stained with either anti-CD4 reagents (APC, Alexa Fluor® 647 and Alexa Fluor® 700) or anti-CD8 (APC-eFluor® 780) prior to fixation. The blue histogram represents staining of unfixed cells and the grey dotted histogram represents staining of fixed cells.



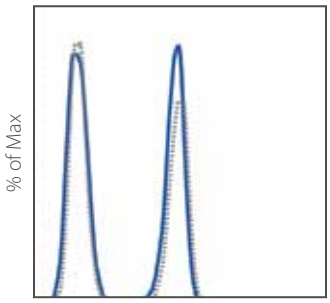
CD4 (SK3) APC



CD4 (OKT4) Alexa Fluor® 647

APC, eFluor® 660 and Alexa Fluor® 647

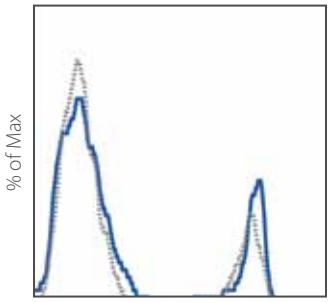
All of these fluorophores are amenable to fixation. While APC shows a 38% reduction in MFI in this example, the resolution of the positive population from the negative, as calculated by the stain index, is unaffected.



CD4 (OKT4) Alexa Fluor® 700

Alexa Fluor® 700

This small organic molecule exhibits no loss of fluorescent signal following fixation.



CD8 (RPA-T8) APC-eFluor® 780

APC-eFluor® 780

This tandem dye retains its fluorescent signal following fixation. Additionally, fixation does not increase the amount of compensation required from the APC detector.

Viability Detection Reagents For The Red Laser

Excluding dead cells from analysis is highly recommended for all flow cytometry experiments in order to minimize the potential artifacts introduced by antibody conjugates which bind nonspecifically to dead cells. eBioscience offers Fixable Viability Dyes for the red laser that are ideal for use with intracellular staining protocols. These are amine-reactive fluorescent reagents that bind free amine groups on proteins. They are not membrane permeable and, therefore, only minimally label live cells. However, dead cells with compromised membranes allow access of the dye to the interior of the cell resulting in a very bright fluorescence of the dead cell population. These dyes are fixable and are retained in cells following fixation and permeabilization steps required for intracellular (IC) staining protocols. Therefore, they are extremely useful in IC protocols where non-specific binding of antibody conjugates can lead to erroneous interpretation of data.

Fixable Viability Dye eFluor® 660: This reagent is detected on a flow cytometer using the same detection filters as those used for APC, eFluor® 660 and Alexa Fluor® 647 (660/20 BP) and cannot be used in combination with these dyes.

Fixable Viability Dye eFluor® 780: This reagent is detected using the standard filter set for APC-eFluor® 780 (780/60 BP) and cannot be used in combination with that dye. We recommend using a cell sample stained only with the Fixable Viability Dye eFluor® 780 to set compensation, as using an APC-eFluor® 780 conjugated antibody as a surrogate will result in gross overcompensation from the detectors for APC and Alexa Fluor® 700. Most cell samples will have an adequate population of dead cells to stain, however, cell death can be induced easily by heat shock of the cell sample.

Ordering Information

Product	Catalog Number	Application Notes	Excitation Laser (nm)	Peak Emission (nm)
Fixable Viability Dye eFluor® 660	65-0864	Permanently labels dead cells	633, 635, 640	660
Fixable Viability Dye eFluor® 780	65-0865	Ideal for intracellular staining protocols	633, 635, 640	780

Filter Recommendations For eBioscience Fluorophores For The Red Laser

Red Laser	Excitation Laser (nm)	Peak Emission (nm)	Dichroic Mirror	Band Pass Filter
APC	633, 635, 640	660	--	660/20
Alexa Fluor® 647	633, 635, 640	668	--	660/20
Alexa Fluor® 700	633, 635, 640	723	685 LP	710/40, 710/50, 720/45
APC-eFluor® 780	633, 635, 640	780	740 LP	780/60

New products are launched regularly. **Discover more at www.eBioscience.com.**

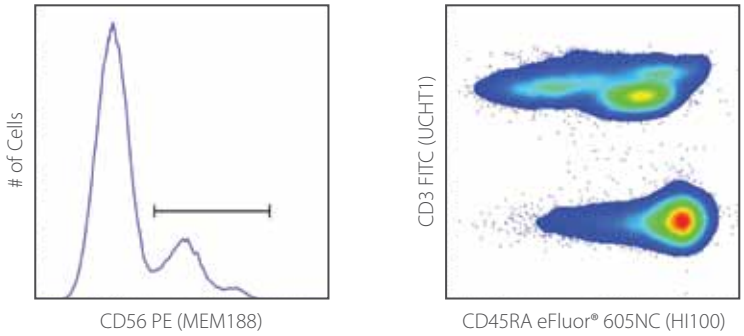
Ten Color Staining Example – *Powered By eFluor®*

■ Violet Laser (405 nm)
CD4 eFluor® 450
CD45RA eFluor® 605NC
CD45RO eFluor® 650NC

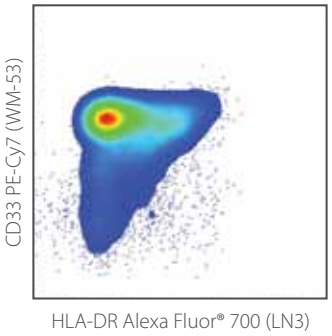
■ Blue Laser (488 nm)
CD3 FITC
CD56 PE
CD19 PerCP-Cy5.5
CD33 PE-Cy7

■ Red Laser (633 nm)
CD8 APC
HLA-DR Alexa Fluor® 700
CD27 APC-eFluor® 780

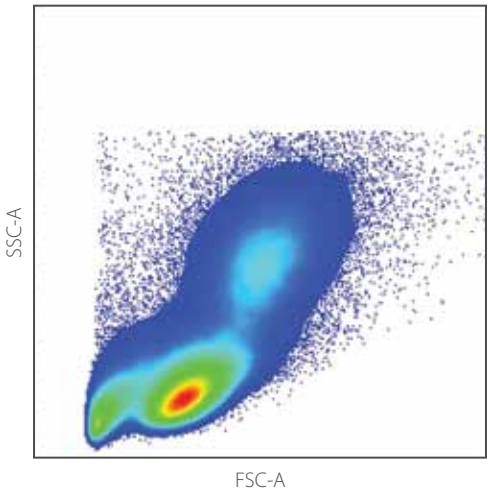
NK(T) Cells



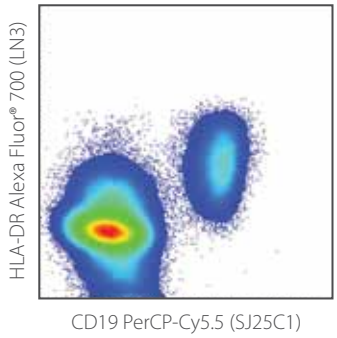
Monocytes



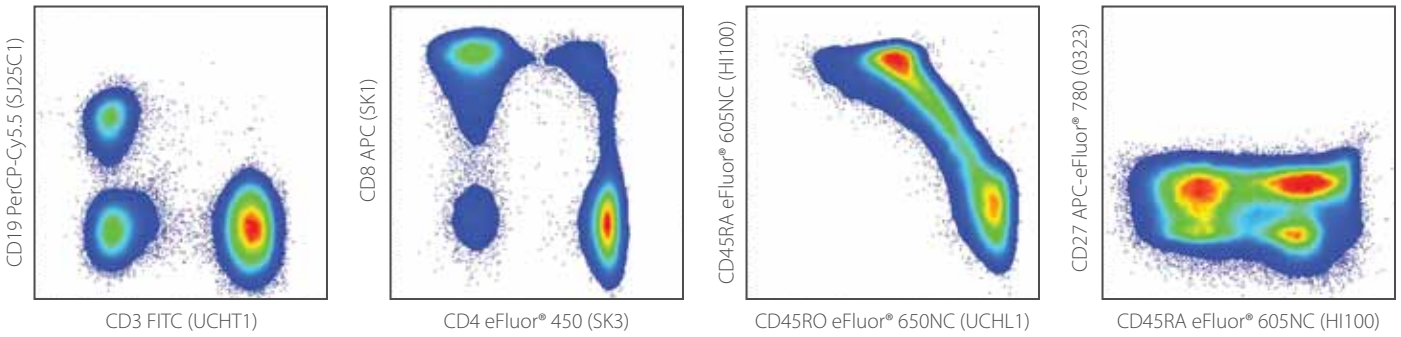
Human PBMC



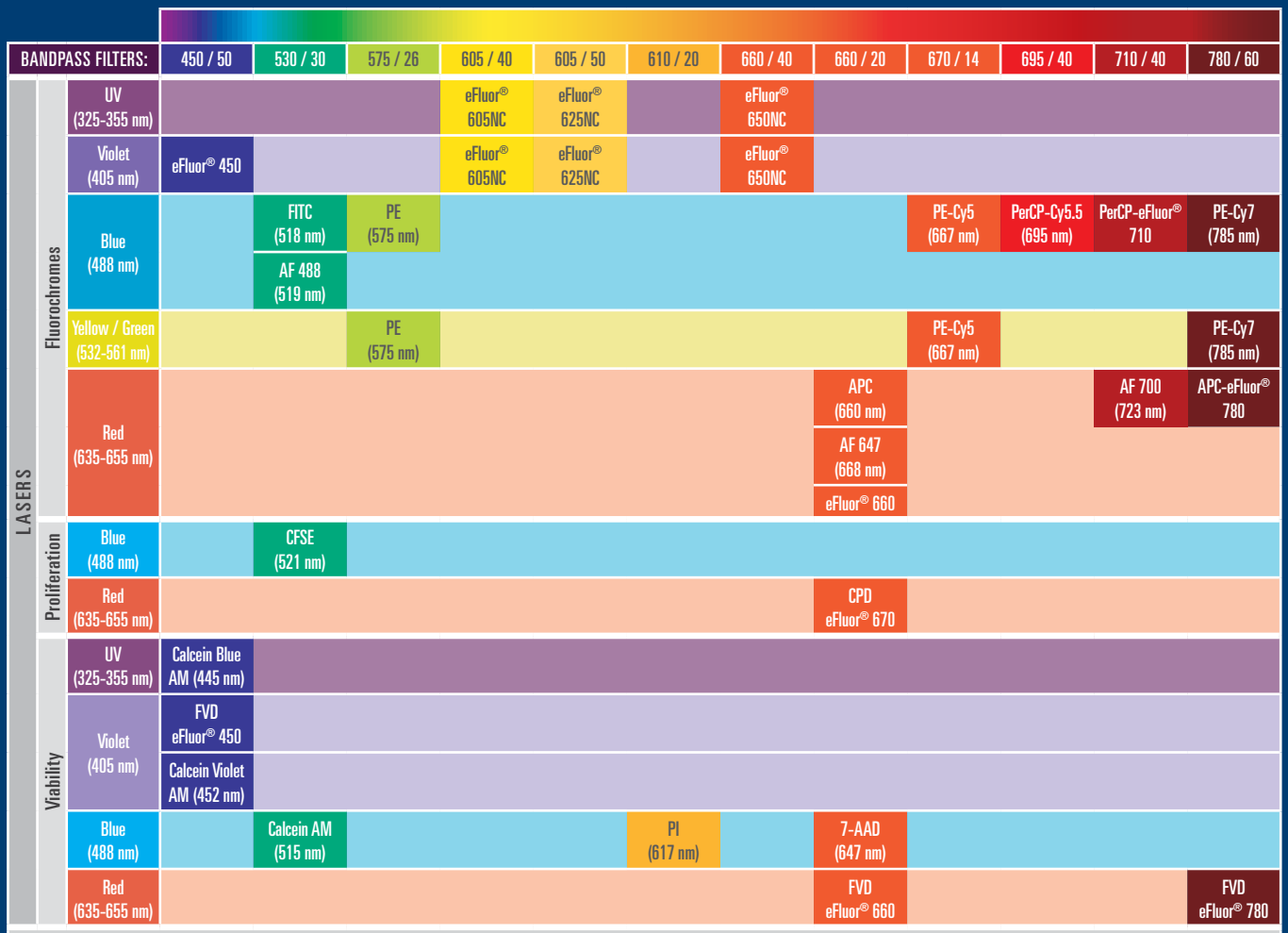
B Cells



T Cells



Possess the Power of the Full Spectrum!



Note: Peak emission for eFluor® dyes is noted in the name. Peak emission for all other dyes is shown in parentheses. Before combining reagents in multicolor experiments, always refer to your specific system configuration.

Product Key: AF = Alexa Fluor®; CPD = Cell Proliferation Dye; FVD = Fixable Viability Dye; PI = Propidium Iodide

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