BioLegend®

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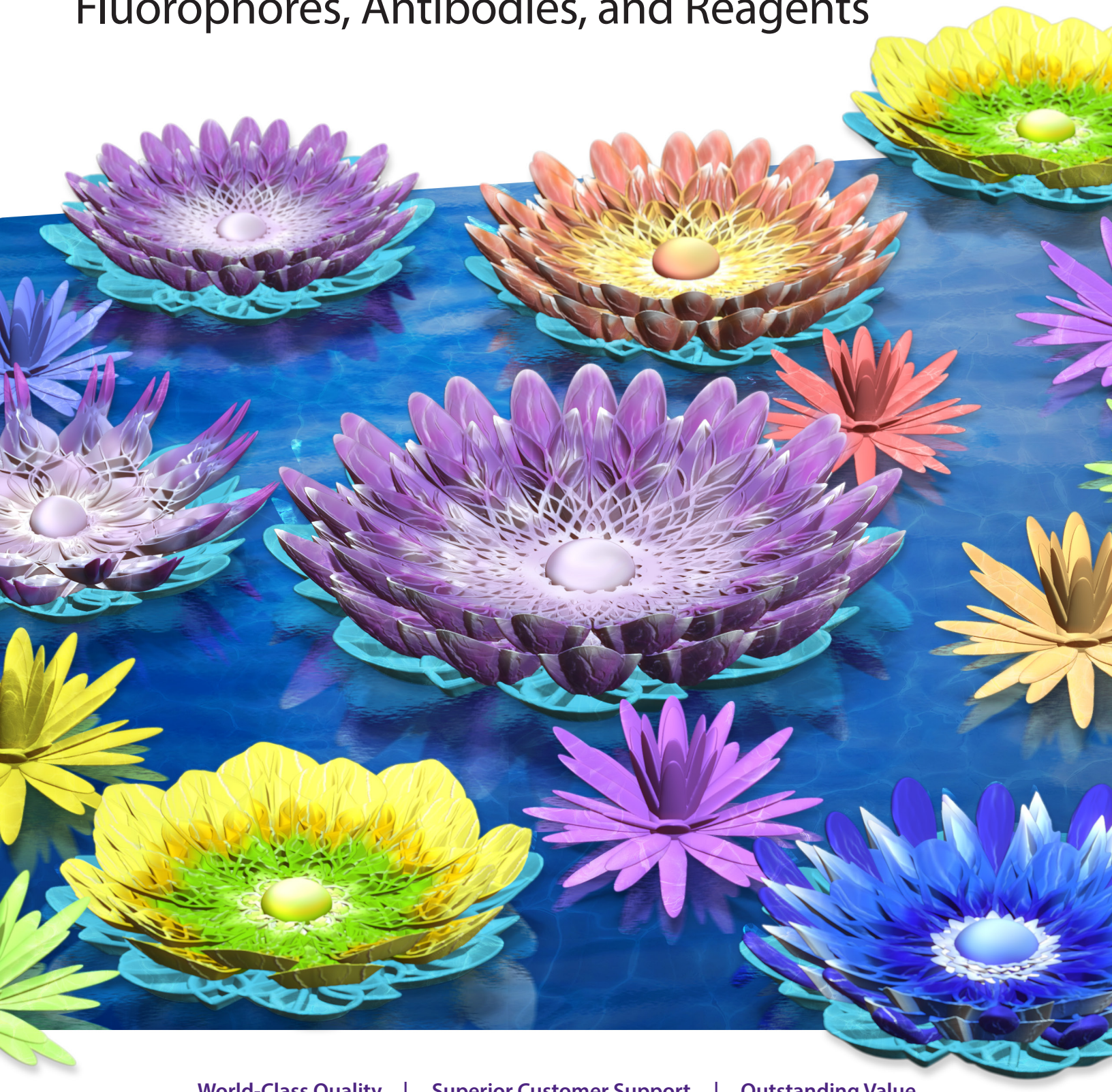
Flow Cytometry

Fluorophores, Antibodies, and Reagents



World-Class Quality | Superior Customer Support | Outstanding Value

Table of Contents

Scientist-Driven Research Solutions

BioLegend is a globally recognized leader in flow cytometry. To advance the capabilities of this application, we provide researchers with reagents of unparalleled quality and foster a creative environment for collaborators. Since our establishment in 2002, we have made our customers and partners our priority by developing the fluorophores, chemical probes, and antibodies needed to support your discoveries. Each individual reagent can be used in applications that accelerate breakthroughs in key research areas like immunology, infectious disease, cancer research, neuroscience, and stem cell development.

Our scientists listen to your feedback and incorporate it at every level of our company, including product development, quality control, emerging technologies, marketing, sales, and technical services. With so many scientists in these key positions, it ensures our support extends beyond our labs and into yours. It is this dedication that has led us to become the most cited flow cytometry reagent provider[1]. Contact us today to discuss your flow cytometry needs and discover why researchers worldwide trust BioLegend.

Technical Services

- Troubleshoot experiments and advise on data analysis
- Provide technical/performance data about reagents
- Optimize experimental design including multicolor flow cytometry panels

Field Application Scientists

- Make virtual or in-person lab visits to demonstrate the utility of applications and reagents
- Review scientific concepts, applications, and techniques with your lab
- Optimize experimental designs including multicolor flow cytometry panels
- Manage custom projects and sales needs

Custom Services Team

- Tailor reagents to your project's needs
- Provide custom conjugations options to 30+ fluorophore and protein formats
- Customize the test size, concentration, and bottling of your reagents
- Offer bulk lots to ensure lot-to-lot consistency
- Provide custom antibody cocktails including lyophilized and dried down products

Customer Service

- Place orders for your reagents and follow-up on their status
- Find information on shipping capabilities
- Check product availability

To contact these US and international groups, visit: biolegend.com/en-us/contact

References:

1. Williams, Rhys. "New leader in the flow cytometry research antibody market." CiteAb. 28 April 2020. blog.citeab.com/antibody-flow-cytometry-market-data/

Quality Leads to Reliability

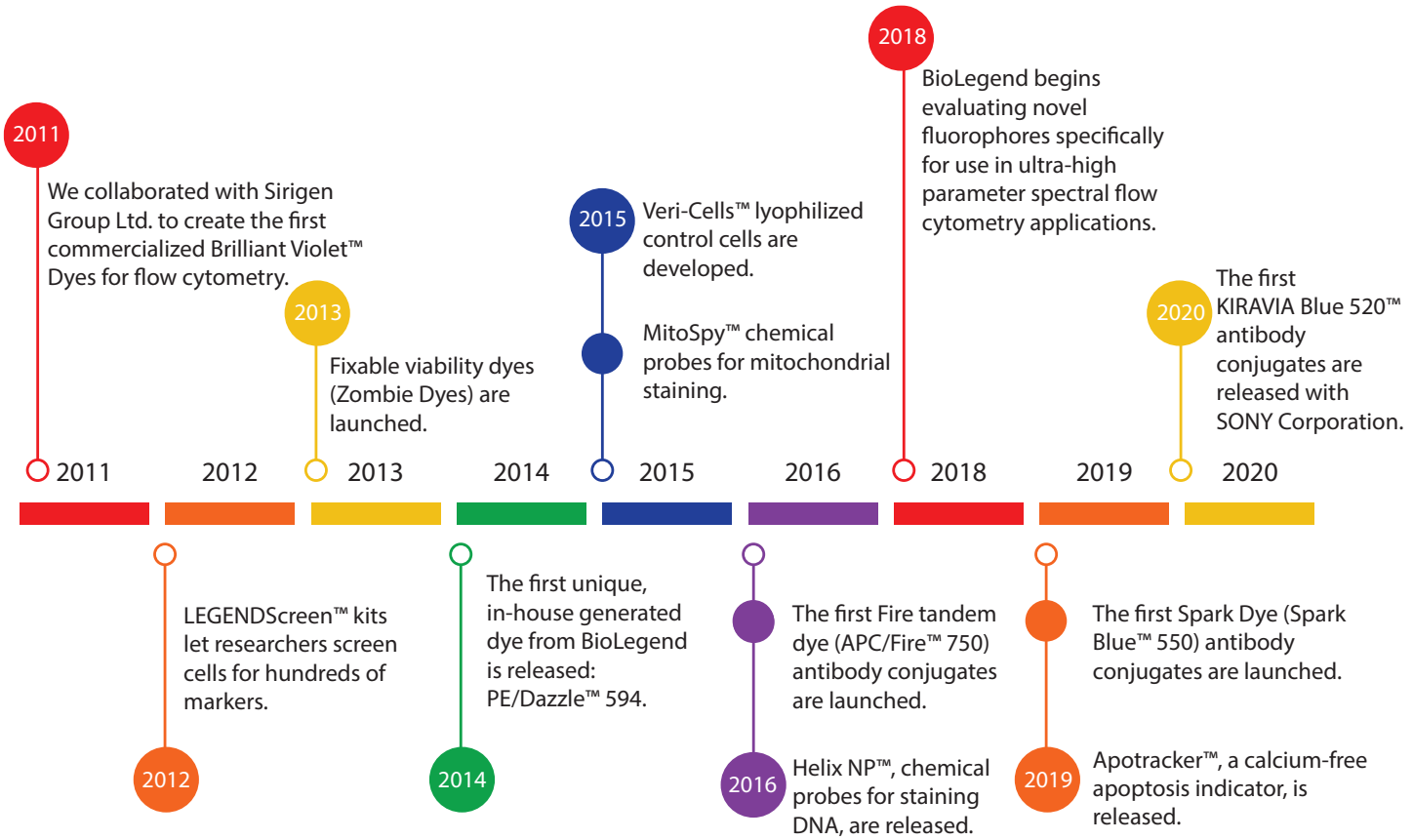
In order to ensure your experimental results are reproducible and reliable, we are continually holding ourselves to a higher standard to improve the quality of our reagents. Our Quality Management System (QMS) is certified through our accredited Notified Body TÜV SÜD to ISO 13485:2016. All of our products undergo industry-leading rigorous quality control (QC) testing to establish the highest level of performance.

Our flow cytometry reagent testing includes:

- Each lot is compared to an internally established "gold standard" to maintain batch-to-batch consistency.
- We test on primary cells rather than artificial samples like cell lines that may overexpress the analyte. Our samples and protocols reflect our customers' experience.
- We test specificity with 1-3 target cell types in single-color or multi-color analysis (including positive and negative cell types). This data can be requested from Technical Services.
- Once specificity is confirmed, each new batch must perform with similar intensity to the in-date reference lot. Brightness (MFI) is evaluated from both positive and negative populations.
- Each batch product is validated by QC testing with a series of titration dilutions.
- Stability studies are performed to guarantee an accurate shelf-life for our products.

A BioLegend Flow Cytometry Product Timeline

We've created innovative reagents for flow cytometry for decades. Take a look back at some of our most important releases over the years.



The Evolution of Flow Cytometry

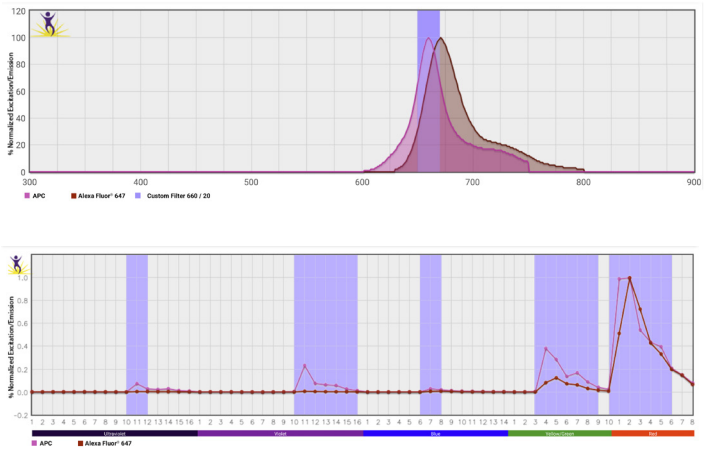
Conventional and Spectral Cytometry

Flow cytometry is an analytical technique used to characterize cells and particles based on light scattering and fluorescent molecule detection. For cell analysis, antibodies and molecular probes conjugated to fluorescent molecules (also referred to as fluorophores or dyes) are most often used for phenotyping of surface and intracellular proteins. When these fluorophores are excited by a particular wavelength of light (like a laser tuned to a specific wavelength), they emit a longer emission wavelength that can be detected in flow cytometers. Conventional flow cytometers rely on a series of mirrors and filters to block, reflect, and transmit emitted signals coming from a fluorophore into individual detectors. In this context, the signal collected in each individual detector constitutes a dedicated signal coming from the fluorescent molecules present.

Technology for both conventional and traditional flow cytometer instrumentation have undergone tremendous advancements in recent years. Traditional cytometers now have an ever-increasing capacity to carry more laser lines and detectors. Even with the improvements in conventional cytometer technology, their optics are designed around directing light at particular defined points in the emission spectra into dedicated detectors. Thus, it is not possible to separate spectrally similar fluorophores that contribute signal to the same filter on a laser.

In contrast, full spectrum flow cytometers capture light emission across many detectors for each fluorophore. Through the use of single-color controls for each fluorophore used, spectral cytometers are capable of generating unique spectral signatures, or "fingerprints" to enable unmixing and identification of unique fluorophores from a multicolor sample[2, 3].

State-of-the-art full spectrum flow instruments, such as the SONY ID7000™ and Cytek® Aurora, are now equipped with photodetectors that capture a wider range of wavelengths. In addition, these newer spectral cytometers eschew the gratings and prisms of original spectral flow cytometers in favor of improved optical filter systems to disperse light. And, the larger number of detectors equipped in spectral flow instruments binned to capture finer segments of wavelengths across the spectrum allows for unprecedented multiparameter capabilities compared to conventional cytometers. For example, conventional cytometers with a red laser often have a 660/20 filter to capture signal from either Alexa Fluor® 647 or APC (allophycocyanin), not both. A conventional cytometer is not capable of distinguishing the signal from these two fluorophores within this one filter set. However, on a spectral cytometer, these two fluorophores can be unmixed from one another and used simultaneously in a panel due to differences in their full spectrum signature collected from detectors for each laser.



Images display the spectras of Alexa Fluor® 647 and APC as compared on a conventional cytometer (top) and a spectral unmixing cytometer (bottom). As the spectral 'fingerprint' of Alexa Fluor® 647 can be distinguished from APC at multiple detectors, these two fluorophores can be unmixed.

Spectra figures above generated using our conventional and spectral cytometry on our flow cytometry tools webpage: biolegend.com/en-us/flow-cytometry-tools

Pairing Highly Specific Antibodies With Novel Fluorophores

High-quality reagents are essential for maximizing the effectiveness of a flow cytometry assay. In particular, flow cytometrists require highly specific antibodies to accurately find their targets and fluorophores that can easily be distinguished from one another. We are industry-leading experts in both antibody and fluorophore development, having built up our antibody library to offer nearly 3,000 clones for our customers across immunology, cancer research, stem cells, and neuroscience targets to provide the most diverse set of research tools for phenotyping and characterizing cell populations.

Fluorophores comprise the other vital arm of flow cytometry reagents, and we've been developing and innovating them since our establishment. From the breakthrough violet laser-excited Brilliant Violet™ dyes created with Sirigen to our newest Spark and Fire Dyes for spectral cytometers, we are helping researchers reach their maximum potential with over 17,000 antibody conjugates for flow cytometry.

References:

2. Bonilla, D.L., et al. 2021. Front Mol Biosci. 7:612801.
3. Nolan J.P. and Condello D. 2013. Curr Protoc Cytom. Chapter 1:Unit 1.27.

Antibodies and Targets at a Glance

Flow cytometry begins with finding the right antibodies for the right specificities. Decades ago, Köhler and Milstein developed the revolutionary techniques that allowed us to create both hybridoma-generated and recombinant monoclonal antibodies. In order to phenotype cell populations, scientists study and detect protein level expressions throughout the cell. Our antibody library helps you target surface, intracellular, and nuclear proteins.

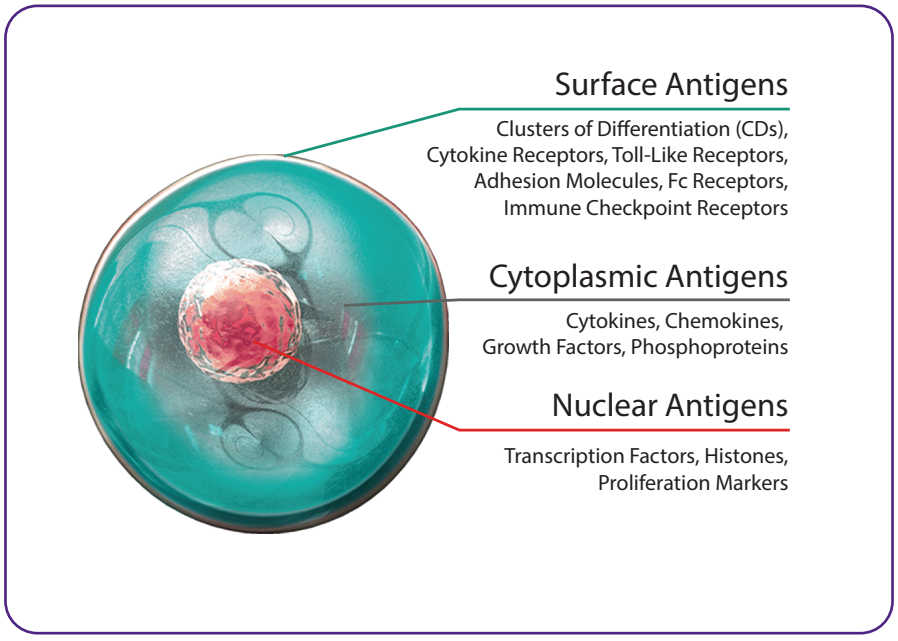
Surface markers make up a large portion of your ability to phenotype in flow cytometry, as combinations of these markers are often used to identify cell populations. For example, T cells are often denoted by expression of the T cell receptor (TCR), CD3, and either CD4 or CD8. In addition, the cell's surface also contains receptors waiting for the proper ligands to bind and initiate a signaling cascade. This includes receptors for cytokines and chemokines; Fc (Fragment crystallizable) receptors that bind to the Fc portions of antibodies; and Toll-like receptors that respond to pathogen-associated molecular patterns.

Intracellular targets can consist of proteins that will be secreted by cells, such as cytokines, chemokines, and growth factors. Detection of these targets often requires treating the cells with protein transport inhibitors like Monensin and Brefeldin A to keep the proteins locked within the cells for staining. Other intracellular targets include phosphoproteins which are vital to signaling pathways and the regulation of downstream protein production.

Finally, nuclear targets can often reveal information on the fate of a cell. Nuclear transcription factors offer signatures unique to specific cell types. In particular, T helper cells are often characterized by their transcription factor expression: Tregs express FOXP3, Th1 cells express T-bet, and Th2 cells express GATA3. Although there is some plasticity between T cell populations in transcription factor expression, these can be early indicators of the phenotype of a T helper cell. The nucleus also houses genetic content like DNA and histones. Histones, in particular, are valued for assessing their epigenetic modifications and methylation sites that can control access to genes.

If you are unable to find an antibody of interest on our website, talk with our Custom Solutions Team (cst@biolegend.com) to discuss the possibility of custom antibody creation. In addition to traditional hybridoma-generated antibodies, we have the capabilities to make recombinant antibodies, with options for partial or full length sequences, host species, and isotypes.

*For surface, intracellular, and nuclear staining, effective staining of antibodies must be complemented by proper buffer usage. See page 16 for a full explanation of our buffer sets.



Fluorophores at a Glance

Our knowledge and expertise with fluorophores, chemistries, and antibody conjugation has helped us become the preeminent experts on flow cytometry. Our scientists are crafting dyes that ignite discoveries by emitting in the far red, fill previously unoccupied spectral spaces, and provide enhanced stability and/or brightness. Explore our fluorophore options below to see what best fits your panel.

Learn more at: biolegend.com/en-us/fluorophore-families

Violet Laser (405 nm)

Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
Pacific Blue™	410	455	450/50	1
Brilliant Violet 421™	405	421	450/50	4
Brilliant Violet 510™	405	510	510/50	1
Spark Violet™ 538*	399	538	-	1
Brilliant Violet 570™	405	570	585/42	1
Brilliant Violet 605™	405	603	610/20	3
Brilliant Violet 650™	405	645	660/20	3
Brilliant Violet 711™	405	711	710/50	4
Brilliant Violet 750™	405	750	780/60	3
Brilliant Violet 785™	405	785	780/60	3

Blue Laser (488 nm)

Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
KIRAVIA Blue 520™	488	520	530/30	3
Alexa Fluor® 488	495	519	530/30	1
FITC	493	525	530/30	1
Spark Blue™ 550*	516	540	-	1
Spark Blue™ 574*	506	574	-	1
PerCP	482	675	695/40	1
PerCP/Cyanine5.5	482	690	695/40	2

Yellow/Green Laser (532, 561 nm)

Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
PE	565	575	585/20	5
Spark YG™ 581*	565	581	-	2
Spark YG™ 593*	565	593	-	3
PE/Dazzle™ 594	565	610	610/20	5
PE/Fire™ 640*	565	639	-	4
PE/Fire™ 700	565	695	710/50	5
PE/Cyanine5	565	670	660/20	5
PE/Cyanine7	565	774	780/60	4
PE/Fire™ 810*	565	806	-	4

Note: PE conjugates can be excited by the blue laser in addition to the yellow/green laser.

Red Laser (633 nm)

Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
APC	650	660	660/20	4
Alexa Fluor® 647	650	668	660/20	4
Spark NIR™ 685*	660	685	-	2
Alexa Fluor® 700	696	719	720/45	2
APC/Cyanine7	650	774	780/60	2
APC/Fire™ 750	650	774	780/60	2
APC/Fire™ 810*	650	807	-	3

*Dyes denoted by an asterisk are recommended to be used on spectral unmixing flow cytometers. They may be compatible with conventional flow cytometers, but end users may need to optimize filter sets.

Fluorophore Families

Spark Dyes

Watch your multicolor panels grow with Spark Dyes, a family of small, synthetic fluorophores. The addition of these expertly-crafted dyes to our portfolio provides researchers with the ability to fill spectral spaces between existing fluorophores, maximizing panel flexibility. Spark Dyes are advantageous due to their relatively narrow emission profile, stability, and solubility. In addition, their synthetic nature means they are not typically sensitive to standard fixatives, including organic solvents used for phospho-flow.

Learn more at: biolegend.com/en-us/spark-dyes

Excitation Laser Legend

Ultraviolet Laser (350 nm)
Violet Laser (405 nm)
Blue Laser (488 nm)
Yellow/Green Laser (532, 561 nm)
Red Laser (633 nm)

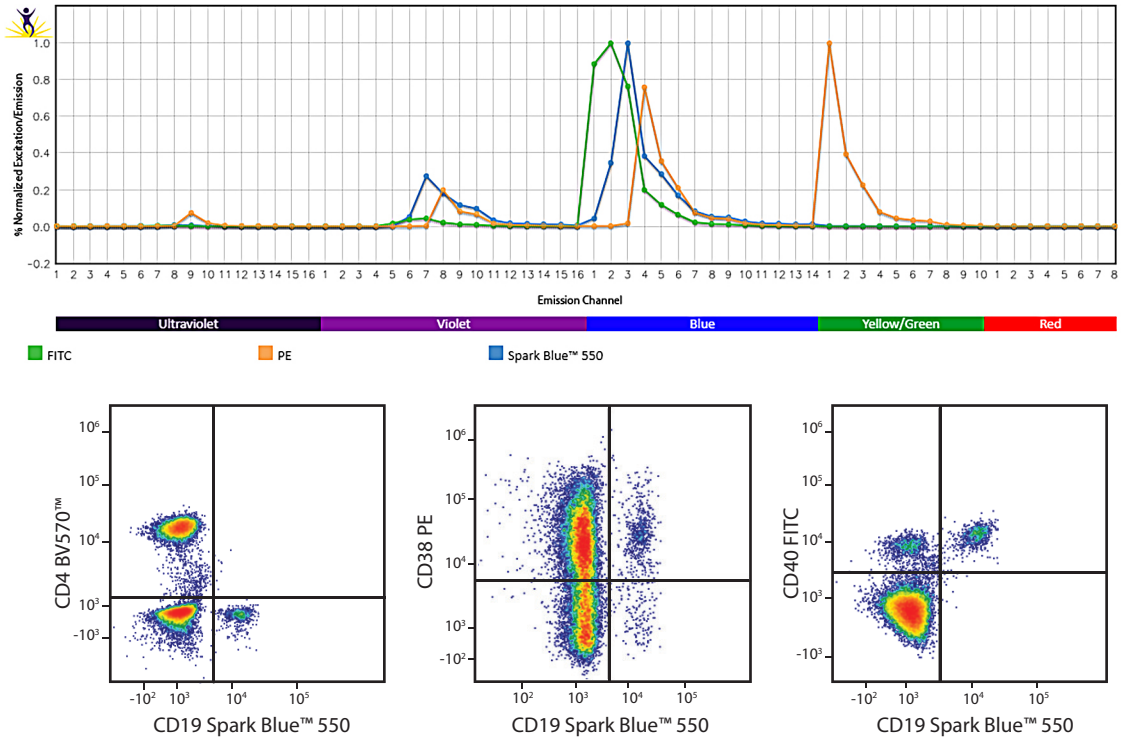
	Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
	Spark Violet™ 538*	399	538	-	1
	Spark Blue™ 550*	516	540	-	1
	Spark Blue™ 574*	506	574	-	1
	Spark YG™ 581*	565	581	-	2
	Spark YG™ 593*	565	593	-	3
	Spark NIR™ 685*	660	685	-	2

*Dyes denoted by an asterisk are recommended to be used on spectral unmixing flow cytometers. They may be compatible with conventional flow cytometers, but end users may need to optimize filter sets.

Product Highlight: Spark Blue™ 550

Discovering New Spectral Niches

Creating new useful fluorophores means finding new spectral spaces for them to occupy. Sometimes this means discovering niches in between established fluorophores that were previously untouched on conventional cytometers. With the help of spectral cytometer's unmixing capabilities, dyes like Spark Blue™ 550 can utilize these newly available spectral niches. Spark Blue™ 550 does not exhibit high spillover with other fluorophores, making it easy to fit into a large panel. It is maximally excited by the 488 nm laser with minimal emission off of the 561 nm laser. Spectrally, it falls between the emissions of FITC and PE, but can easily be used in the same panel as these fluors have minimal spillover considerations.



Human whole blood was stained with anti-CD19 Spark Blue™ 550, anti-CD4 BV570™, anti-CD38 PE, and anti-CD40 FITC antibodies. Samples were unmixed on a Cytek® Aurora Cytometer using compensation beads and cells. All plots are gated on lymphocytes.

Fire Dyes

Our Fire Dyes are tandem fluorophores that push the limits of flow capabilities by expanding into spectral niches previously unused in conventional cytometry. In addition, we also offer Fire Dyes with enhanced stability and brightness properties for existing channels. Ignite your next discovery with these dyes specifically designed for spectral cytometry and other advanced flow cytometry applications.

Learn more at: biolegend.com/en-us/fire-dyes

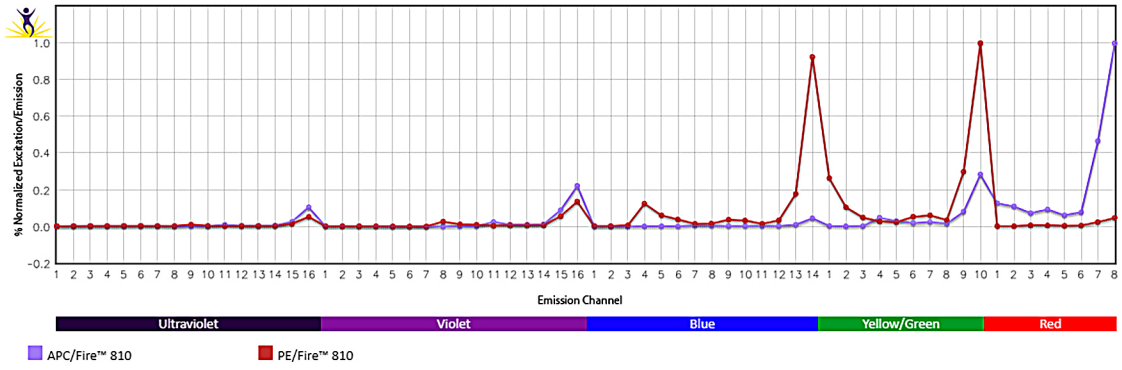
	Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
	PE/Fire™ 640*	565	639	-	4
	PE/Fire™ 700	565	695	710/50	5
	PE/Fire™ 810*	565	806	-	4
	APC/Fire™ 750	650	774	780/60	2
	APC/Fire™ 810*	650	807	-	3

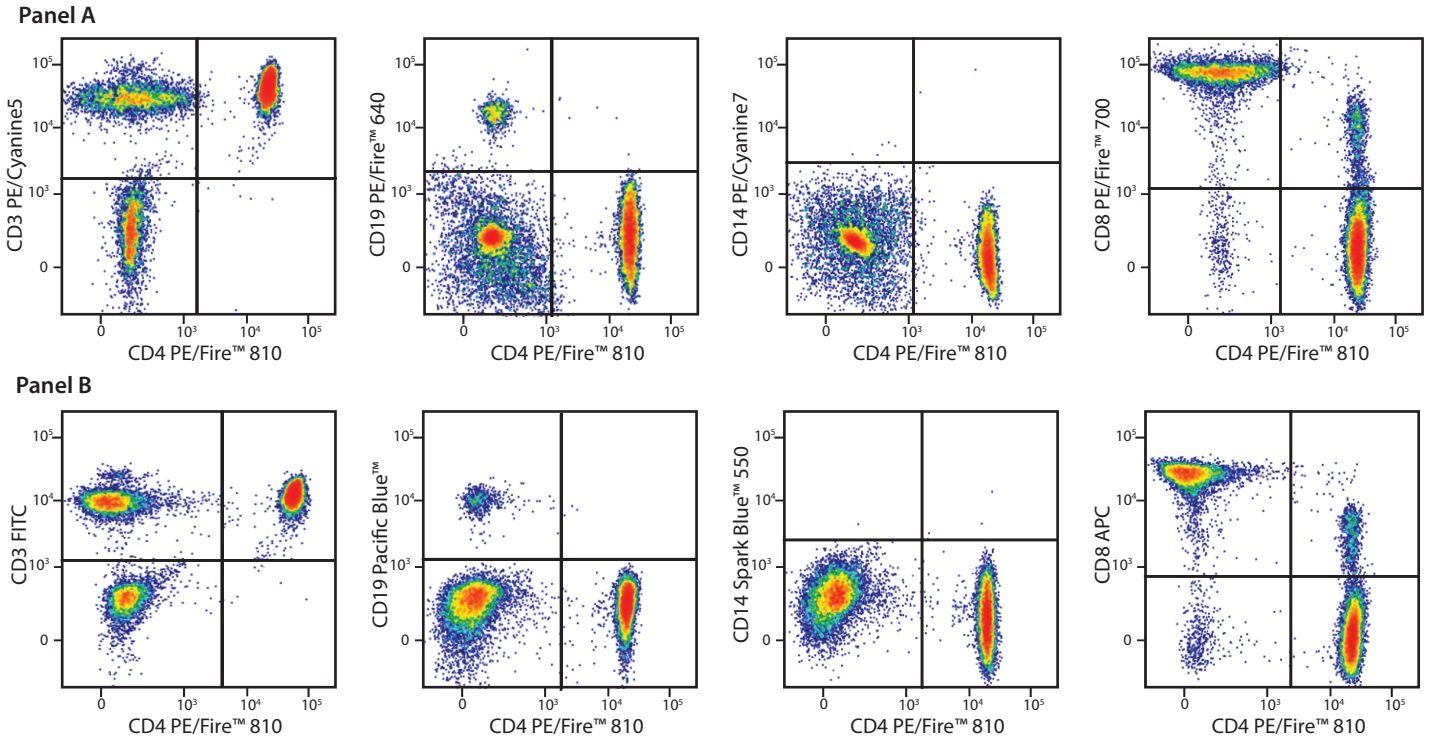
*Dyes denoted by an asterisk are recommended to be used on spectral unmixing flow cytometers. They may be compatible with conventional flow cytometers, but end users may need to optimize filter sets.

Product Highlight: APC/Fire™ 810 & PE/Fire™ 810

Expanding Into the Far Red

Along with PE/Fire™ 810, APC/Fire™ 810 expands the range of our spectral detection farther into the infrared than any of our previous fluorophores. Due to their far-red emissions, APC/Fire™ 810 and PE/Fire™ 810 typically only exhibit significant spillover with one another on a 5-laser Cytek® Aurora (UV/V/B/YG/R) or SONY ID7000™. Given that these two dyes emit far outside of the autofluorescent range and have few neighboring fluorophores, they can be utilized for antigens of ubiquitous or varying levels of expression. These characteristics make these dyes convenient to add into pre-existing panel designs with minimal adverse impact.





Human peripheral blood lymphocytes stained with a multicolor panel with either a high (Panel A) or low (Panel B) degree of spectral complexity and overlap. CD4 vs. CD8 plots are also gated on the $CD3^+$ lymphocyte population.

Brilliant Violet™ Dyes

Brilliant Violet™ antibody conjugates are an innovative class of research reagents, providing more options for your multicolor flow cytometry panels and better results. Maximize the capacity of your violet laser with our large selection of directly labeled Brilliant Violet™ antibody conjugates.

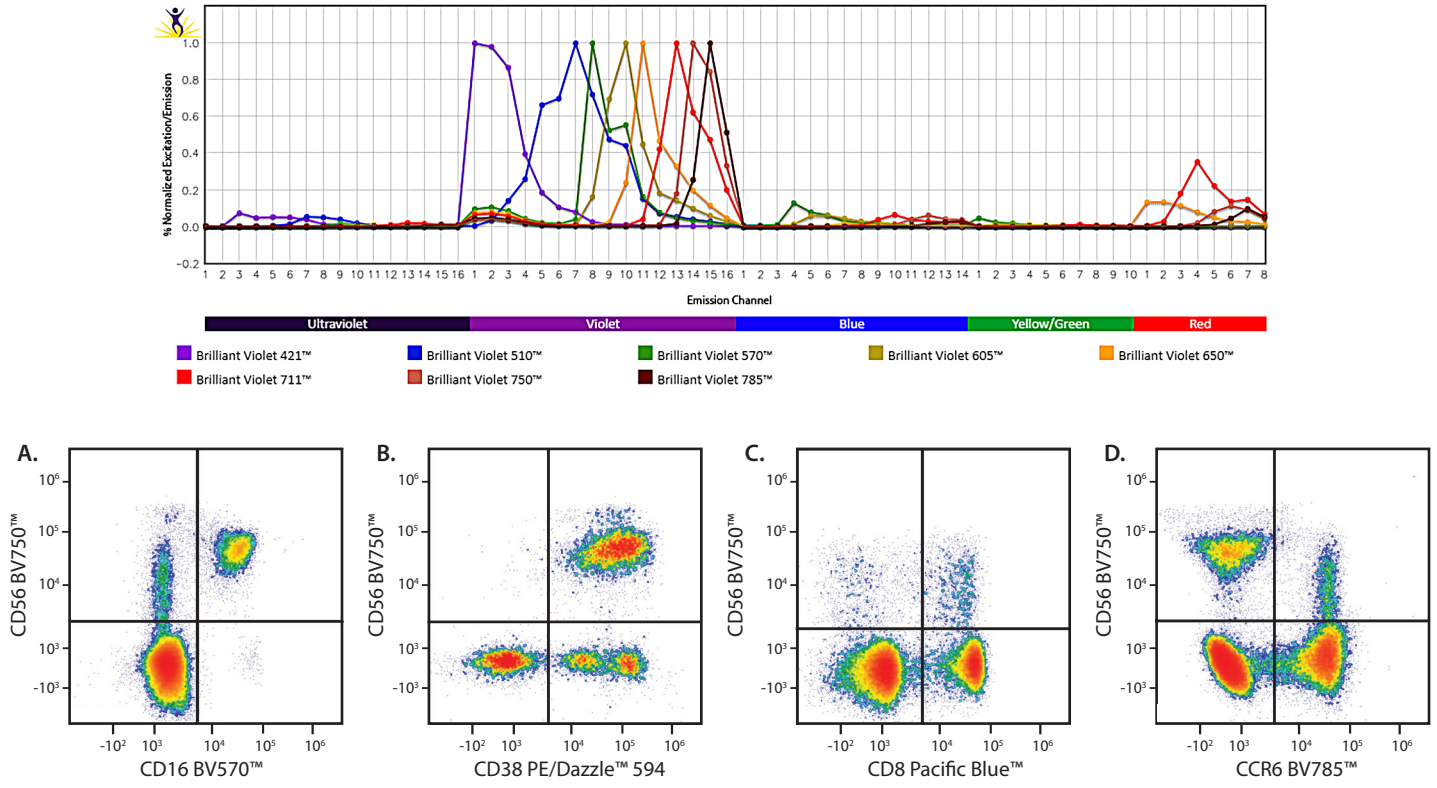
Learn more at: biolegend.com/en-us/brilliant-violet

	Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
	Brilliant Violet 421™	405	421	450/50	4
	Brilliant Violet 510™	405	510	510/50	1
	Brilliant Violet 570™	405	570	585/42	1
	Brilliant Violet 605™	405	603	610/20	3
	Brilliant Violet 650™	405	645	660/20	3
	Brilliant Violet 711™	405	711	710/50	4
	Brilliant Violet 750™	405	750	780/60	3
	Brilliant Violet 785™	405	785	780/60	3

Product Highlight: Brilliant Violet™ 750

Adding to the Brilliant Violet™ Family

Brilliant Violet 750™ is a tandem dye based on the Brilliant Violet 421™ polymer core. It provides additional options for the violet laser, particularly for those with either a spectral detection cytometer or a cytometer with a decagon configuration for the violet laser. Alternatively, it can be used in place of BV785™ on a standard violet laser octagon configuration.



Human whole blood was stained with 21 different antibody conjugates and analyzed on the Cytek® Aurora Spectral Flow Cytometer. Gates were applied based on the plots as follows: A/D: lymphocyte scatter profile, B. $CD3^-/CD20^-$ cells, C. $CD3^+$ cells.

KIRAVIA Dyes™

KIRAVIA is a coined term meaning "sparkling" in Japanese. This class of dyes employs a unique organic backbone that separates fluorophores to minimize quenching effects, thus allowing optimal and higher fluorophore to protein (F:P) ratios, surpassing what is possible with direct conjugations of single fluorophores like FITC.

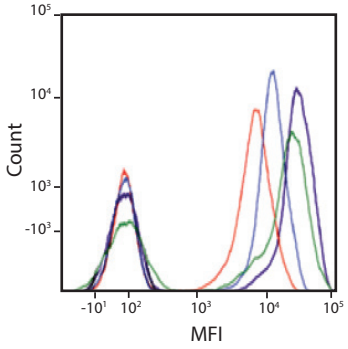
	Fluorophore	Excitation Max (nm)	Emission Max (nm)	Recommended Filter	Brightness
	KIRAVIA Blue 520™	488	520	530/30	3

Learn more at: biolegend.com/en-us/kiravia

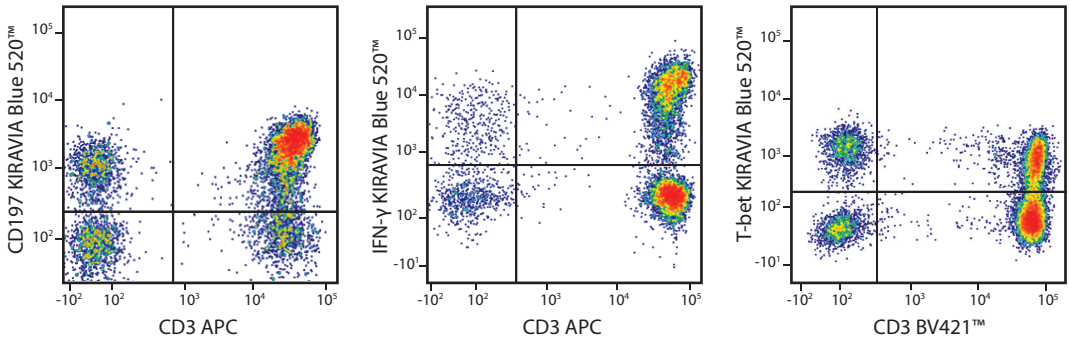
Product Highlight: KIRAVIA Blue 520™

Improving on Existing Fluorophores

As new technology is discovered, scientists can innovate with new fluorophore chemistries and characteristics. For example, FITC is one of the earliest discovered fluorophores and has been incorporated into flow panels for decades. However, FITC itself is not a particularly bright fluorophore and may have trouble providing resolution for lowly expressed markers. KIRAVIA Blue™ 520 is spectrally identical to FITC, but offers nearly twice the brightness of a FITC conjugate. Its enhanced brightness allows it to successfully resolve surface, intracellular, and nuclear targets.



Anti-human CD4 (clone SK3), conjugated to FITC (red), Alexa Fluor® 488 (blue), BD Horizon™ Brilliant Blue 515 (green), or KIRAVIA Blue 520™ (purple) was used to stain human lysed whole blood. This was compared to unstained cells (black).



(Left) Human lysed whole blood was surface stained with anti-human CD3 APC (clone UCHT1) and anti-human CCR7 (clone G043H7) KIRAVIA Blue 520™. (Middle) PMA/ionomycin-stimulated (6 hours) human PBMCs stained with anti-human CD3 (clone UCHT1) APC and anti-IFN-γ (clone 4S.B3) KIRAVIA Blue 520™. (Right) Human PBMCs were stained with anti-human CD3 (clone UCHT1) BV421™ and anti-human T-bet (clone 4B10) KIRAVIA Blue 520™.

Apoptosis and Cell Health Detection

Cell health is important to researchers for multiple reasons: they may want to assess the viability and integrity of samples; they may be studying molecular pathways of apoptosis (programmed cell death); or they may be looking to track a cell's division and migration. To provide researchers with flexibility for their projects, we provide reagents tailored to each of these goals.

Learn more at: biolegend.com/en-us/cell-health-tools

Live/Dead Indicators

Within a sample, it can be important to assess the viability and general health of a cell. Dying/dead cells can create debris that lead to false positive staining within a flow cytometry experiment. A majority of the reagents in this category (*e.g.* Propidium Iodide, 7-AAD, DAPI, and Helix NP™ (non-permeant) dyes) function on the concept that intact membranes of living cells help to limit the binding of a fluorescent dye or chemical probe. Fixation and permeabilization reagents (*e.g.* alcohols and paraformaldehyde) can unwind and denature DNA, dislodging DNA-binding dyes and giving false negative results. Simultaneously, free dye can find its way into previously healthy cells that have now been fixed/permeabilized and give a false positive result.

To avoid this issue, we provide Zombie Dyes, which bind the amine groups present on proteins. While live cells are stained to a low degree due to surface proteins, dead cells will stain much more brightly due to the high abundance of internal proteins that can be stained once membranes are compromised.

Learn more at: biolegend.com/en-us/live-dead

Excitation Laser Legend

	Ultraviolet Laser (350 nm)
	Violet Laser (405 nm)
	Blue Laser (488 nm)
	Yellow/Green Laser (532, 561 nm)
	Red Laser (633 nm)

Fixable Viability Dyes

	Chemical Probe	Excitation Max (nm)	Emission Max (nm)	Recommended Filter
	Zombie UV™	360	459	450/50
	Zombie Violet™	400	423	450/50
	Zombie Aqua™	382	516	510/50
	Zombie Yellow™	396	572	585/42
	Zombie Green™	490	515	530/30
	Zombie Red™	600	624	610/20
	Zombie NIR™	719	746	780/60

DNA-Binding Dyes

	Chemical Probe	Excitation Max (nm)	Emission Max (nm)	Recommended Filter
	DAPI	360	460	450/50
	CytoPhase™ Violet	369	440	450/50
	Helix NP™ Blue	430	470	450/50
	Helix NP™ Green	495	519	530/30
	Propidium Iodide	493	620	610/20
	7-AAD	546	647	650 LP
	DRAQ5™	598	680	695 LP
	DRAQ7™	633	695	695 LP
	Helix NP™ NIR	640	660	660/20

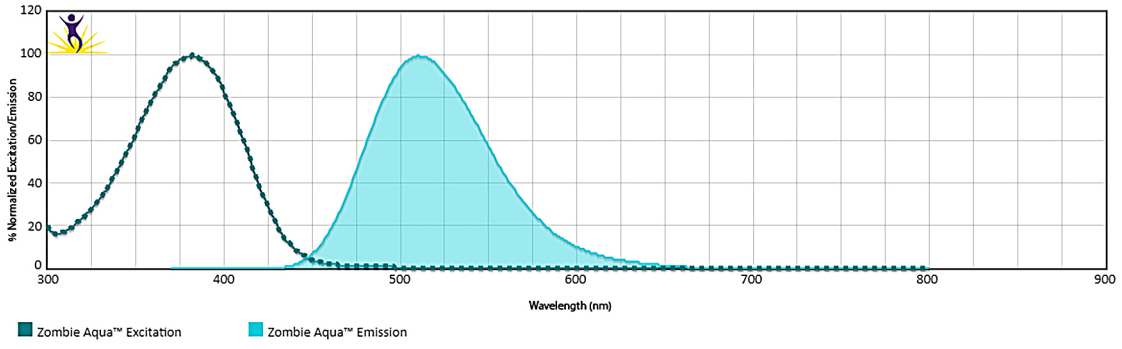
LP indicates a long pass filter.

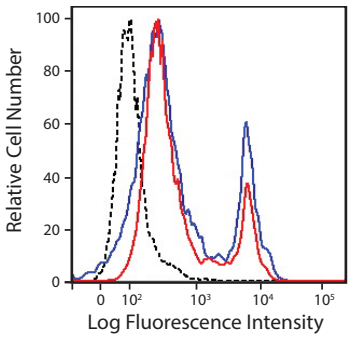
When Propidium Iodide intercalates with DNA, its excitation and emission max become 535 and 617 nm respectively.

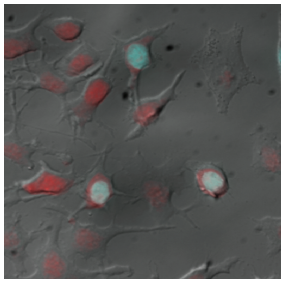
When DRAQ5™ intercalates with DNA, its excitation and emission max become 633 and 695 nm respectively.

Product Highlight: Zombie Aqua™

In flow cytometry, Zombie Aqua™ is typically detected in the Brilliant Violet 510™ filter (510/50) for the violet laser of conventional instruments. The ability of this dye to remain bound to primary amines through the fixation processes provides easily distinguished dying or unhealthy cell populations. In addition, the versatility of this reagent allows it to be used in microscopy assays for viability assessment.







(Left) One day old splenocytes were stained with Zombie Aqua™ and analyzed without fixation (blue) or analyzed after fixation and permeabilization (red). Cells alone without Zombie Aqua™ staining are indicated in black. (Right) HeLa cells were treated with 20% EtOH for 20 seconds, washed twice with PBS, and then were left to recover for five minutes with cell culture media at 37°C. The cells were stained with Zombie Aqua™ (1:1000) (cyan) for 15 minutes and then fixed with 1% paraformaldehyde (PFA) for ten minutes. Nuclei were counterstained with DRAQ5™ (red) for five minutes. The image was captured with 40X objective.

Apoptosis Indicators

Apoptosis is a type of programmed cell death used by cells for self-elimination. Apoptotic cells do not release their cellular contents and are quickly engulfed by phagocytic cells, and therefore avoid triggering unnecessary inflammatory responses. Since many physiological functions rely on the proper elimination of cells, dysregulation of apoptosis can contribute to diseases like cancer and neurodegeneration.

We provide antibodies to proteins involved in apoptotic pathways, including the Bcl-2 family, TNF family, death ligands and receptors, and caspases. Historically, Annexin V has been utilized to detect early stages of apoptosis since this protein has high affinity for phosphatidylserine (PS). On healthy cells, PS is typically only found on the inner/cytosolic membrane. In the early stages of apoptosis, PS begins to appear on the outer membrane as the cell becomes compromised, allowing it to be detected by chemical probes like Annexin V or Apotracker™.

Learn more at: biolegend.com/en-us/apotracker

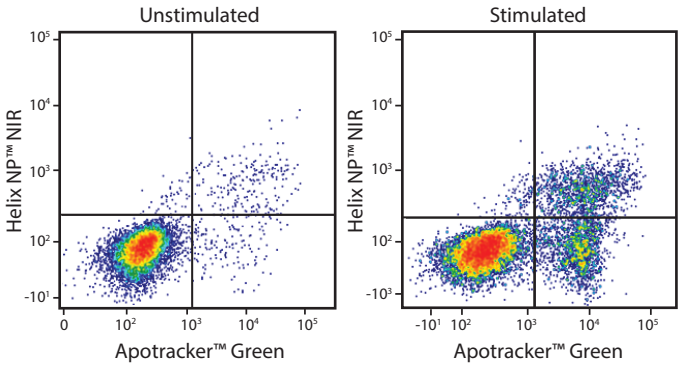
Apoptosis Indicators

	Chemical Probe	Excitation Max (nm)	Emission Max (nm)	Recommended Filter
	Apotracker™ Green	500	520	530/30
	Annexin V Conjugates*	-	-	-
	Annexin V Binding Buffer	-	-	-

*We offer over a dozen formats of Annexin V conjugated to a fluorophore.

Product Highlight: Apotracker™ Green

Discover an innovative method to detect apoptotic cells with Apotracker™. Many traditional apoptosis detection reagents like Annexin V require the use of calcium-containing buffers. However, calcium has been shown to potentially affect cell viability. Apotracker™ circumvents this issue as it requires no special buffer exchange steps, shortening your overall protocol. In addition, the signal from Apotracker™ is preserved after fixation with mild PFA fixatives, making it easy to use for both flow cytometry and microscopy applications.



Unstimulated (left) and CD95-stimulated (right) Jurkat cells were stained with Apotracker™ Green (Apo-15) and Helix NP™ NIR. Cells were stained for 10-15 min in FACS buffer followed by two washes prior to analysis.

Vitality Indicators

Other functional probes can also assess live/dead status depending on their cellular vitality. Long-term cell tracking probes like CFSE and Tag-it Violet™ help to indicate a cell's health as it relies on its esterase activity. The simplest example of cell vitality, however, is Calcein-AM, Calcein Red-AM and Calcein Violet-AM. These are simple fluorogenic esterase probes that are passively cell-permeant and then subsequently retained by the cell when the probe becomes charged upon conversion. The brighter the signal, the more abundant the esterase activity and the healthier the cell. As cells die, the esterase activity will be reduced until no activity remains. These probes can be used to track the cells for as long as the signal persists. However, they are considered short-term trackers since they do not bind to internal proteins like CFSE does, and most cells will eventually start to pump out these probes naturally.

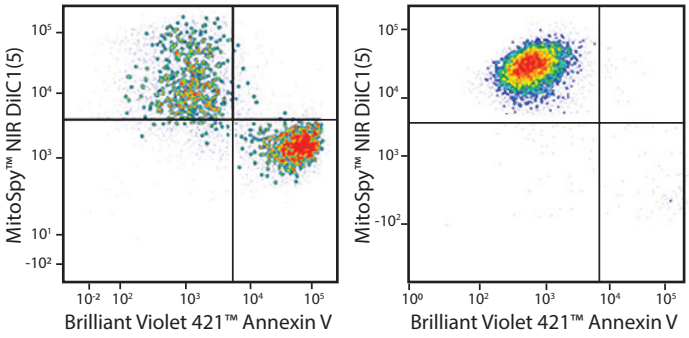
Learn more at: biolegend.com/en-us/cell-health-and-proliferation

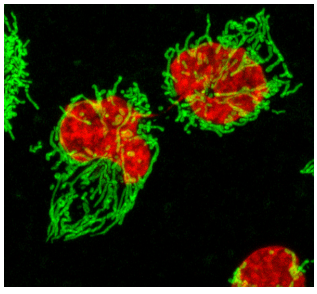
Vitality Indicators

	Chemical Probe	Excitation Max (nm)	Emission Max (nm)	Recommended Filter
	Calcein Violet-AM	400	452	450/50
	Tag-it Violet™	395	455	450/50
	Calcein-AM	494	514	530/30
	CFSE	492	517	530/30
	MitoSpy™ Green FM	490	516	530/30
	Calcein Red-AM	560	574	585/20
	MitoSpy™ Orange CMTMRos	551	576	585/20
	MitoSpy™ Red CMXRos	577	598	610/20
	MitoSpy™ NIR DilC1(5)	638	658	660/20

Product Highlight: MitoSpy™ NIR

Mitochondrial respiration and the polarization of the mitochondrial membrane are also indicators of cell health. Probes like MitoSpy™ Orange, MitoSpy™ Red, and MitoSpy™ NIR are live cell-permeant fluorogenic probes that localize to the mitochondrial membrane based on its strong polarization in healthy, respiring cells. When used in conjunction with other probes for cell health, like Annexin V, Apotracker™, or impermeant nucleic acid stains, the neutralization of potential across the mitochondrial membrane can be one of the first indications that a cell is entering apoptosis. MitoSpy™ Green localizes to the mitochondria independent of membrane potential and thus should be used as a measure of mitochondrial mass in cells as opposed to general cell health.





(Top) Jurkat cells were treated (left) or un-treated (right) with 1.0 µg/ml of LEAF™ purified anti-human CD95. At the end of incubation, cells were washed twice (once with PBS then with Annexin V Binding Buffer), then were stained with 5 nM of MitoSpy™ NIR DilC1(5) and BV421™ Annexin V for 15 minutes at 37°C in Annexin V Binding Buffer. After incubation, cells were washed and resuspended with Annexin V Binding Buffer. (Bottom) HeLa cells were stained with 0.25 µM CytoPhase™ Violet dye (red) for 60 minutes at 37°C. Then 20 nM of MitoSpy™ NIR DilC1(5) (green) was added for an additional 30 minutes at 37°C. Image courtesy of the Biophotonics Core Facility at the Salk Institute.

Cell Cycle Analysis

The cell cycle is a series of steps that cells must undergo for replication. Cells divide in response to stimuli such as growth factors, cytokines, and specific antigens. This response must be tightly regulated, since improper cell proliferation can lead to tumor growth or developmental problems. Given their affinity for DNA, some chemical probes can be used to determine the cell cycle status of a cell. While Propidium Iodide, DAPI, DRAQ5™, DRAQ7™, CytoPhase™ Violet, and Helix NP™, can all be used to stain fixed cells for cell cycle analysis, only CytoPhase™ Violet and DRAQ5™ can be used to assess DNA content of healthy, unfixed populations since they are capable of permeating membranes of live cells. For additional cell cycle analysis tools, we also provide antibodies for proliferation markers like Ki-67 and cyclins.

Learn more: biolegend.com/en-us/cell-cycle

BrdU Kits

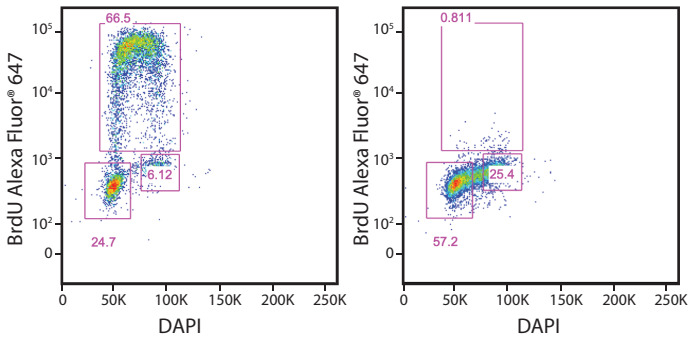
	Description	Excitation Max (nm)	Emission Max (nm)	Recommended Filter
	Phase-Flow™ BrdU Kit FITC	493	525	530/30
	Phase-Flow™ BrdU Kit Alexa Fluor® 647	650	668	660/20

Product Highlight: Phase-Flow™ Alexa Fluor® 647 BrdU Kit

BrdU (bromodeoxyuridine) assays identify proliferating cells. BrdU is a nucleotide analog that can be given to cells in culture media or via direct injection into an animal to be incorporated into the newly replicating DNA of dividing cells. This reveals which of the cells achieved division during the time of the BrdU pulse. It can be combined with other common flow cytometry stains to reveal more information about a cell's DNA content, phenotype, and response to mitogens. Phase-Flow™ Kits conveniently bundle together the necessary reagents to measure BrdU incorporation by flow cytometry.

Kit Contents:

- BrdU pulsing solution
- Anti-BrdU antibody
- Buffers
- DNAse (lyophilized)
- PBS (Ca^{2+}/Mg^{2+})
- 7-AAD
- DAPI



Ramos cells loaded with BrdU for 2 hours (left panel) or left as no load controls (right panel) stained using the Phase-Flow™ Alexa Fluor® 647 BrdU Kit and DAPI.

Flow Cytometry Buffers

Sample Preparation

Before the staining process even begins, we offer specialized buffers and solutions to help with your workflow. In particular, Lymphopure™ is a density gradient-based media that separates mononuclear cells from red blood cells and granulocytes. We also provide red blood cell lysing solutions with and without fixative to fit your experimental needs.

Sample Preparation Solutions

Buffer	Function
Lymphopure™	Density gradient media for easy mononuclear cell (PBMC) isolation.
RBC Lysis Buffer (10x)	Concentrated buffer for red blood cell lysis. Contains ammonium chloride, potassium carbonate, and EDTA.
RBC Lysis/Fixation Solution (10x)	Concentrated buffer for red blood cell lysis and fixation of remaining leukocytes.

Fixation Buffers

Buffer	Function
Fixation Buffer	Paraformaldehyde-based buffer used to fix cells.
FluoroFix™ Buffer	Gentler fixation buffer with a lower percentage of paraformaldehyde specialized for use with tandem dyes.

Storage Buffers

Buffer	Function
Cyto-Last™ Buffer	Specially formulated buffer for prolonged storage of cytokine-producing cells.

Surface Staining

Cell surface markers are often straightforward to detect and do not require any specialized buffers or solutions. For surface staining, we provide a ready-to-use Cell Staining Buffer that can be used for antibody incubations and washes. Some exceptions like Annexin V require the presence of calcium in its staining buffer. Our Apotracker™ chemical probe can be used to stain phosphatidylserine in the absence of calcium, allowing you to use traditional Cell Staining Buffers.

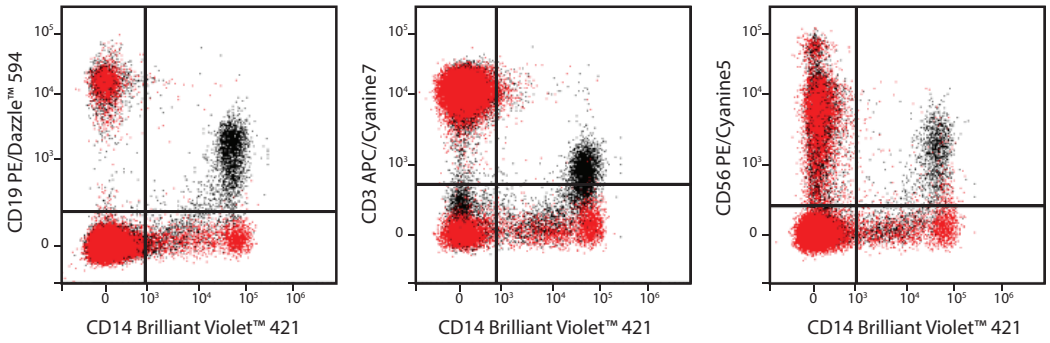
Antigen presenting cells, particularly monocytes and macrophages, can exhibit non-specific binding of molecules that can raise the background signal of your samples. Fc receptors recognize and detect the Fc portion of an antibody, which would initiate a series of innate immune responses *in vivo.* And, monocytes and macrophages have the ability (through a currently unknown mechanism) to bind certain components of the chemical structures in dyes like PE/Cyanine5. For both of these forms of non-specific binding, we provide blocking solutions in the form of TruStain FcX™ and True-Stain Monocyte Blocker™. Taken together, these buffers can help decrease background noise and improve your signal to noise ratios.

Surface Staining Buffers

Buffer	Function
Cell Staining Buffer	Ready-to-use, reliable buffer for cell staining and washing.
Annexin V Binding Buffer	Calcium-containing buffer formulated for Annexin V staining.

Blocking Buffers

Buffer	Function
TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody	Blocks Fc receptors on mouse cells.
Human TruStain FcX™	Blocks Fc receptors on human cells.
True-Stain Monocyte Blocker™	Minimizes the non-specific binding of certain classes of dyes to monocytes and macrophages.



Human peripheral blood mononuclear cells were either untreated (black) or treated with True-Stain Monocyte Blocker™ (red) and stained with CD14 (clone HCD14) Brilliant Violet 421™ and CD19 (clone HIB19) PE/Dazzle™ 594 (left), CD3 (clone UCHT1) APC/Cyanine7 (middle), or CD56 (clone 5.1H11) PE/Cyanine5 (right).

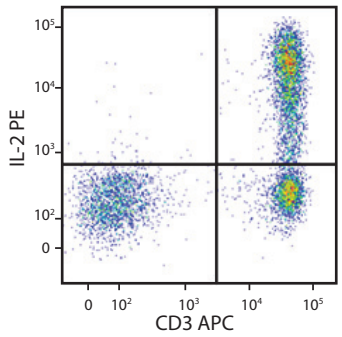
Intracellular Staining

Intracellular targets require additional buffers and solutions for robust detection. Prior to staining, the cells need to be fixed and permeabilized so that the antibodies can bind to these intracellular epitopes. We provide a variety of buffers optimal for staining cytokines, nuclear antigens, and phosphoproteins.

Cytokine Detection

Intracellular flow cytometry can be used to identify the production of cytokines or other cytoplasmic proteins within phenotypically defined populations. Detection of cytokines may first require activation or stimulation of cells and treatment with a protein transport inhibitor such as Monensin or Brefeldin A to block the release of cytokines.

For optimal cytokine detection, we provide a Cyto-Fast™ Fix/Perm Buffer Set. It was developed following rigorous in-house testing to provide optimal staining for intracellular cytokines and other cytoplasmic proteins, like Granzyme B or Perforin.

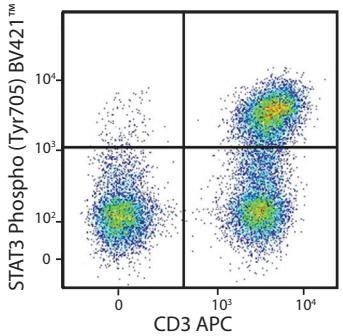


PMA + ionomycin stimulated (3 hours) human peripheral blood lymphocytes (in the presence of monensin) were fixed and permeabilized with Cyto-Fast™ Fix/Perm Buffer Set, then stained with anti-CD3 (UCHT1) APC and anti-IL-2 (clone MQ1-17H12) PE.

Phosphorylated Target Detection

Phosphorylation of protein targets play critical roles for signal transfer and can result in the activation or deactivation of downstream proteins. The detection of phosphorylated proteins using intracellular flow cytometry can be tricky and often requires an alcohol-based permeabilization for the antibody to bind to the phospho-epitope.

True-Phos™ Perm Buffer is recommended to permeabilize cells for phosphoprotein staining. It is a methanol-based perm buffer that can be used in conjunction with our fixation buffer for optimal fixation and permeabilization.



Human peripheral blood stimulated with Recombinant human IL-6 (Cat. No. 570802) for 15 minutes was treated with RBC Lysis/Fixation Solution, permeabilized with True-Phos™ Perm Buffer, and stained with anti-CD3 APC and anti-STAT3 Phospho (Tyr705) Brilliant Violet 421™.

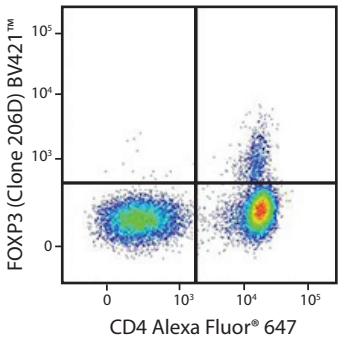
Intracellular Staining Buffers

Buffer	Function
Cyto-Fast™ Fix/Perm Buffer Set	Buffer set for optimal staining of cytokines and other cytoplasmic proteins.
True-Phos™ Perm Buffer	Methanol-based permeabilization buffer for phospho-protein staining.

Nuclear Antigen Detection

Nuclear protein detection includes the identification of transcription factors associated with specific cell types or differentiation and signaling pathways. For example, T regulatory cells are commonly identified by the expression of cell surface markers, CD4 and CD25, and the FOXP3 transcription factor. Nuclear antigen detection often requires a harsher permeabilization method in order to permeabilize the nuclear membrane and release DNA and protein interactions.

For this purpose, we provide True-Nuclear™ Transcription Factor Buffer Set which offers optimal staining for nuclear proteins, like transcription factors, and improved stability for tandem dyes that have been used to stain surface molecules.



Human peripheral blood lymphocytes were surface stained with anti-CD4 Alexa Fluor® 647, treated with the True-Nuclear™ Transcription Factor Buffer Set, then stained with anti-FOXP3 (clone 206D) BV421™.

Nuclear Staining Buffers

Buffer	Function
True-Nuclear™ Transcription Factor Buffer Set	Buffer set for optimal staining of transcription factors.

Learn more about our buffers: biolegend.com/en-us/flow-buffers

Additional Flow Cytometry Reagents

Veri-Cells™

Accurate, Reproducible Lyophilized Human Control Cells for Flow Cytometry

Veri-Cells™ lyophilized human control cells provide accurate and reliable controls to monitor assay performance and variability for longitudinal studies. Our Veri-Cells™ products include controls for immunophenotyping, immunodeficiency, cellular activation, or CyTOF® studies.

Veri-Cells™ Features:

- Excellent long-term stability
- Validated for expression of over 150 surface markers
- Variety of products removes the need to set up stimulation or obtain alternate control samples
- Consistent and reliable—each lot is analyzed for a select number of phenotyping markers and compared to a gold standard to ensure consistency

Veri-Cells™ Products:

- Activated (Cytokine) PBMC
- Activated (Surface) PBMC
- CD34 PBMC
- CD4 Low PBMC
- Heavy Metal (Ta) PBMC
- Leukocytes
- PBMC
- Phospho PBMC (MAPK/ERK Pathway)

Can't find the control cells you need? We offer custom Veri-Cells™ products and lot sizes. Contact our Custom Solutions Team to learn more at cst@biolegend.com.

Learn more: biolegend.com/veri-cells

LEGENDScreen™

Human and Mouse PE Kits to Screen Cell Surface Molecules

LEGENDScreen™ is the most cost-effective way to test over 250 directly-conjugated antibodies and has been incorporated into more advanced applications, like Infinity Flow. Our mouse and human kits can be used to screen cell lines and primary cells (PBMCs, bone marrow-derived cells, and cells isolated from tissues such as spleen and lymph node). The workflow is simple and easy: reconstitute the antibodies, add your cells, collect the samples, and analyze. Cell staining is done directly in the plates, so no additional transfer of reconstituted antibody is required.

LEGENDScreen™ Features:

- Large selection of specificities per kit. The Human PE Kit has 361 cell surface markers plus 10 isotype controls. The Mouse PE Kit has 255 cell surface marker antibodies plus 11 isotype controls.
- Fast and easy-to-follow protocol.
- Directly conjugated antibodies at pre-titrated optimal concentrations provide reliable results.
- Complete kits with staining buffer, fixation buffer, and plate sealers.

Learn more: biolegend.com/en-us/legendscreen

Flow Cytometry Web Tools

With so many new fluorophores, antibodies, and instruments, understanding how to perform your flow cytometry experiment can be difficult. We've created over a dozen web tools to help you at every stage of your workflow. Explore each one to prepare for your experiments.

Plan out your experiments. Utilize our popular Spectra Analyzers to study the excitation and emission properties of commercially available fluorophores. For those using spectral unmixing flow cytometers, the Aurora Spectra Analyzer is ideal for displaying the spectra of fluorophores as captured by a 5-laser Aurora instrument from Cytek®.

In addition, you can utilize our Panel Builders to visualize the available reagents for your targets, choose the right fluorophore/antibody combinations, and share panels with colleagues or BioLegend's scientists.

Explore our web tools: biolegend.com/en-us/flow-cytometry-tools

View our protocol videos: biolegend.com/en-us/video-library

Perform your experiments with ease. When it is time to set your experiments in motion, you can pull up our helpful protocols or watch step-by-step tutorials to guide your work.

Analyze and optimize your results. Once your final protocol step is run, you can begin to delve into your results. If you run into difficulty, you can explore our troubleshooting guide or seek out guidance from our scientific experts by getting in touch with our Technical Services team.

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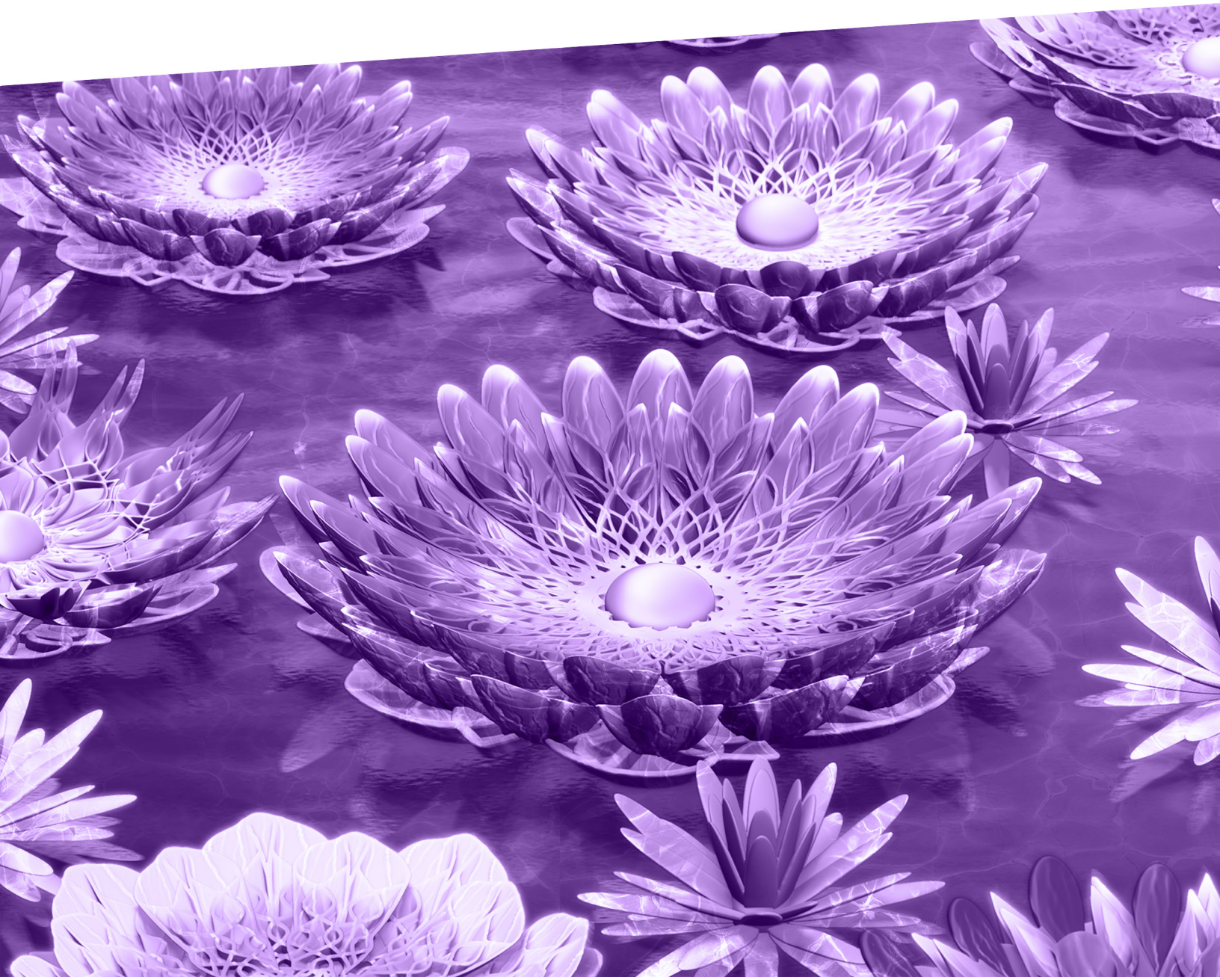

02-0006-04

BioLegend products are manufactured in an ISO 13485:2016-certified facility to ensure the highest quality standards.