Products for Flow Cytometry

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Flow Cytometry Laser Lines for CF® Dyes

Visible spectrum

Far-red

Near-infrared

Laser Line	CF® Dye	λ_{Ex} (nm)	λ_{Em} (nm)	Spectrally similar to	Advantages
UV (355 nm)	CF®350	347	448	Alexa Fluor® 350, AMCA, DyLight® 350	• Brightest blue fluorescent conjugates for 350 nm excitation • Highly water soluble and pH-insensitive
Violet (405 nm)	CF®405S	404	431	Alexa Fluor® 405, Cascade Blue®, DyLight® 405	• Better compatibility with common instruments • Highly water soluble and pH-insensitive
	CF®405M	416	452	BD Horizon™ V450, eFluor® 450, Pacific Blue®	• More photostable and less spill over in the 525/50 green channel than Pacific Blue® dye
	CF®405L	413	547	Pacific Orange®	• Long stokes shift allows multicolor detection from violet laser excitation
	CF®430	426	498	Pacific Green®	• More photostable than spectrally similar dyes • Compatible with AmCyan channel
	CF®440	440	515	Alexa Fluor® 430	
	CF®450	450	538	Unique spectral properties	• 405 nm-excitable green dye with unique spectral properties
Blue (488 nm)	CF®488A	490	515	Alexa Fluor® 488, Cy®2, DyLight® 488, FAM, FITC, Fluorescein	• Yields more specific antibody conjugates and less spill over fluorescence in the red channel than Alexa Fluor® 488 • Extremely photostable, highly water soluble, and pH-insensitive
	CF®503R	503	532	ATTO 488	• Alternative green dye for multispectral detection or FRET
	CF®514	516	548	Alexa Fluor® 514	• Green dye that can be distinguished from CF®488A by spectral unmixing • Extremely photostable, highly water soluble, and pH-insensitive
Blue (488 nm), Green (532 nm)	CF®532	527	558	Alexa Fluor® 532, ATTO 532	• Significantly brighter than Alexa Fluor® 532
	CF®543	541	560	Alexa Fluor® 546, Tetramethylrhodamine (TAMRA)	• Significantly brighter than Alexa Fluor® 546 • Highly water soluble and pH-insensitive
Blue (488 nm), Green (532 nm), Yellow (561-568 nm)	CF®550R	551	577	Unique spectra	• Orange/red dye ideal for multispectral detection or FRET
	CF®555	555	565	Alexa Fluor® 555, ATTO 550, Cy®3, DyLight® 549, TRITC	• Brighter than Cy®3 • Comparable to Alexa Fluor® 555
Green (532 nm), Yellow (561-568 nm)	CF®568	562	583	Alexa Fluor® 568, ATTO 565, Rhodamine Red	• Optimized for the 568 nm line of the Ar-Kr mixed-gas laser • Brighter and more photostable than Alexa Fluor® 568
	CF®570	568	591		
	CF®583	583	606	Cy®3.5, Texas Red®	• Less background than Cy®3.5 and Texas Red® • Ideal for FRET when paired with R-PE
	CF®583R	586	609	Cy®3.5, Texas Red®	• Brighter than Cy®3.5 and Texas Red® • Ideal for FRET when paired with R-PE
	CF®594	593	614	Alexa Fluor® 594, ATTO 594, DyLight® 594	• Yields the brightest conjugates among spectrally similar dyes • Extremely photostable
Red (633-647 nm)	CF®620R	617	639	LightCycler® Red 640	• Highly fluorescent, highly water soluble, and extremely photostable
	CF®633	630	650	Alexa Fluor® 633, Alexa Fluor® 647, Cy®5, DyLight® 633	• Yields the brightest antibody conjugates among spectrally similar dyes • Far more photostable than Alexa Fluor® 647
	CF®640R	642	662	Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649	• Has the best photostability among dyes with Cy®5-like spectra • Highly fluorescent, highly water soluble, and pH-insensitive
	CF®647	650	665	Alexa Fluor® 647, Cy®5, DyLight® 649	• Brighter than Cy®5 • Highly water soluble and pH-insensitive
	CF®660C	667	685	Alexa Fluor® 660	• Much brighter and more photostable than Alexa Fluor® 660 • Highly water soluble and pH-insensitive
	CF®660R	663	682	Alexa Fluor® 660	• Brighter than Alexa Fluor® 660 • The most photostable 660 nm dye • Highly water soluble and pH-insensitive
	CF®680	681	698	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	• The brightest among spectrally similar 680 nm dyes • Superior signal-to-noise ratio in immunostaining • Highly water soluble and pH-insensitive • Compatible with LI-COR Odyssey® System
	CF®680R	680	701	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	• The most photostable 680 nm dye • Suitable for labeling nucleic acids and small biomolecules • Highly water soluble and pH-insensitive • Compatible with LI-COR Odyssey® System
Near-IR (785-808 nm)	CF®700	695	700	Alexa Fluor® 700, DyLight® 700	• Exceptionally bright and stable • Patented PEGDye™ dye for superior performance
	CF®750	755	777	Alexa Fluor® 750, Cy®7, DyLight® 750, IRDye® 750 APC-Alexa Fluor® 750	• Exceptionally bright and stable • Highly water soluble without bearing excessive charge • Better signal-to-noise ratio compared to APC-Alexa Fluor® 750 tandem dye with 633 nm excitation
	CF®770	770	797	DyLight® 800, IRDye® 800CW	• Exceptionally bright and stable • Highly water soluble without bearing excessive charge • Superior signal-to-noise for conjugates • Compatible with LI-COR Odyssey® System
	CF®790	784	806	Alexa Fluor® 790	
	CF®800	797	816	Indocyanine Green	
	CF®820	822	835	DY-820	

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Alexa Fluor, Cascade Blue, Pacific Blue, Pacific Green, DyLight and Texas Red are registered trademarks of Thermo Fisher; ATTO dyes are products of ATTO-TEC GmbH; BD Horizon is a trademark of BD Biosciences; Cy is a registered trademark of Cytiva; eFluor is a registered trademark of eBioscience; IRDye and Odyssey are a registered trademark of LI-COR Bioscience; LightCycler is a registered trademark of Roche Applied Science.

Primary Antibodies and Other Bioconjugates

Biotium offers a wide selection of primary antibodies and biomolecules conjugated to our bright and photostable CF® Dyes, as well as R-phycoerythrin (R-PE), allophyocyanin (APC), PerCP, and biotin. Visit www.biotium.com for a full listing of fluorescent conjugates for flow cytometry.

Growing selection of over 1000 mAbs

- Comprehensive library of recombinant monoclonal mouse and rabbit antibodies
- Choice of 13 bright and photostable CF® Dyes
- Other fluorophores available including R-PE, APC, PerCP, HRP, AP, or Biotin
- Isotype control antibodies available conjugated to the same dyes and labels
- BSA-free purified antibodies ready to use for Mix-n-Stain™ labeling

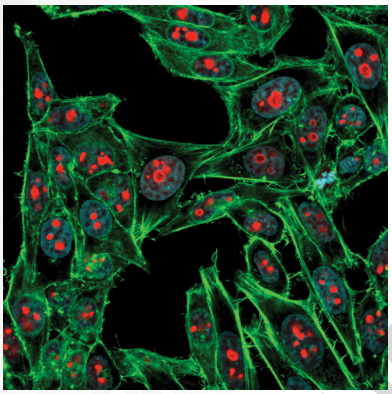


Figure 1. HeLa cells stained with CF®555 monoclonal anti-nucleolin (clone 364-5) (red), CF®488A phalloidin (green), and Hoechst (blue).

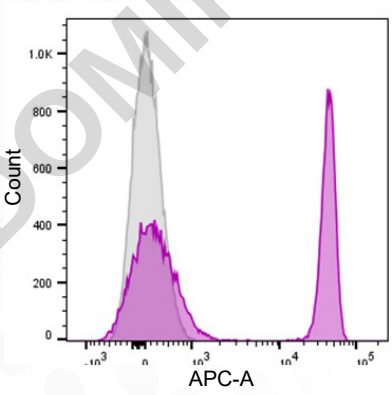


Figure 2. Surface staining of human PBMC with CD4 (EDU-2) CF®640R conjugate (magenta) compared to unstained cells (gray).

Primary Antibody Sizes and Formats

Format	Concentration	Size
CF® Dye conjugates (13 colors)	0.1 mg/mL	100 or 500 uL
Biotin, HRP, or AP conjugates	0.1 mg/mL	100 or 500 uL
R-PE, APC, or PerCP conjugates	0.1 mg/mL	250 uL
Purified, with BSA	0.2 mg/mL	100 or 500 uL
Purified, BSA-free (Mix-n-Stain™ Ready)	1 mg/mL	50 uL

Anti-Tag and Anti-Hapten Antibodies
Mouse Monoclonal Anti-GFP
Rabbit Anti-RFP
Rabbit Anti-GST
Rabbit Anti-HA Tag
Mouse Monoclonal Anti-6X His Tag
Rabbit Anti-Myc Tag
Rabbit Anti-V5 Tag
Rabbit Anti-FLAG Tag
Mouse Monoclonal Anti-Biotin
Mouse Monoclonal Anti-Fluorescein

Other Bioconjugates	Application
Annexin V	Apoptosis (phosphatidylserine) detection (see p. 9)
Dextrans & BSA	Fluid phase endocytosis tracer
Streptavidin	Detection of biotinylated probes
Biotin	Biotinylated probes, and biotin

Looking for another conjugation?

If you are looking for a product not listed on our website, please contact **techsupport@biotium.com** and let us know. We may be able to add it as a new product, or perform a custom conjugation for you.

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Antibody Labeling Kits

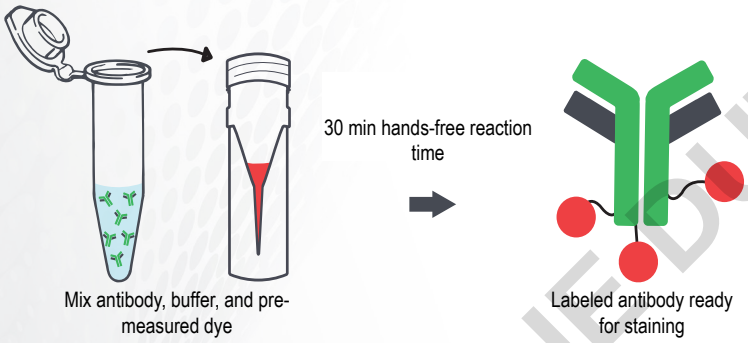
Mix-n-Stain™ Antibody Labeling Kits

Mix-n-Stain™ antibody labeling kits dramatically simplify the process of preparing fluorescently-labeled antibodies. Simply mix your antibody with the reaction buffer and desired fluorophore provided with the kit. The resulting conjugates offer comparable performance to commercially available pre-labeled antibodies (Fig. 1). Because the labeling is covalent, the conjugates are stable for long-term storage, and ideal for multi-color imaging and multiplex flow cytometry.

Mix-n-Stain™ kits feature Biotium's CF® Dyes, which have advantages in brightness and photostability compared to other fluorescent dyes. A variety of other labels are also available.

We also offer Mix-n-Stain™ Nanobody Labeling Kits which allows rapid and optimal labeling of single-chain nanobodies with CF® Dyes or biotin without a purification step.

Mix-n-Stain™ kits are also available in a maxi size for labeling up to 1 mg of IgG antibody with CF® Dyes or other dyes. See the tables on page 5 for a complete listing of available Mix-n-Stain™ Antibody Labeling Kits.



Overview of CF® Dye Mix-n-Stain™ labeling protocol; Mix-n-Stain™ protocols vary for fluorescent proteins (ie, R-PE, APC), tandem dyes, and enzymes.

Mix-n-Stain™ Advantages

- Rapid and efficient labeling with minimal hands-on time
- Available with 29 CF® Dyes, R-PE, APC, PerCP, or tandem dyes
- Other label options: Biotin & FITC, HRP, AP, and GOx
- 100% yield with no purification required
- Labeling tolerates BSA, gelatin, ascites fluid, and Tris
- Kits available for labeling as little as 1 ug and up to 1 mg of IgG antibody
- Labeling kits for nanobodies also available

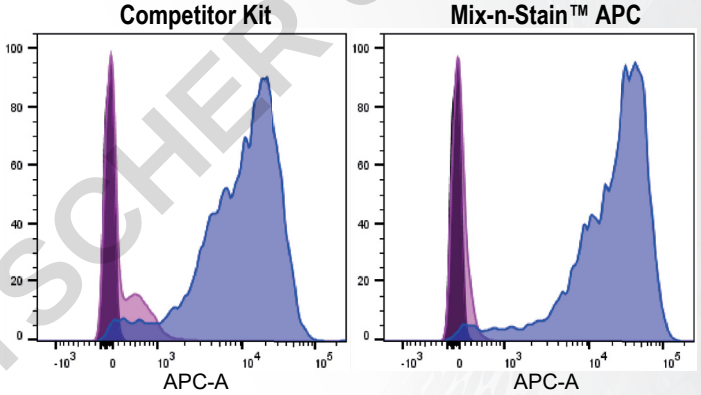


Figure 1. An anti-mouse antibody was labeled with APC using either the Mix-n-Stain™ APC Labeling Kit, or a competitor's APC labeling kit. The antibodies were used to stain Jurkat cells that had been incubated with or without mouse anti-CD3 primary antibody. The Mix-n-Stain™ labeled antibody showed no background on no-primary control cells (pink) but a strong signal on CD3-labeled cells (blue).

CF® Dye SE Protein Labeling Kits

CF® Dye SE Protein Labeling kits allow you to label your antibody of interest with one of our many bright and photostable CF® Dyes or biotin. The difference is that while the Mix-n-Stain™ kits come optimized for fast and consistent labeling of small amounts of antibody, the SE Protein Labeling Kits are designed for 3 labelings of up to 1 mg protein with removal of free dye after labeling. The kits also allows the user to adjust the amount of dye and protein to optimize the degree of labeling. See the tables on page 5 for a complete listing of available SE protein labeling kits.

CF® Dye SE Protein Labeling Kit Features

- Kits that allow more flexibility for labeling optimization
- Choice of 19 CF® Dye colors or biotin
- Includes reagents for 3 x 1 mg labeling reactions with purification
- Includes instructions for determining the degree of labeling (DOL)

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CF Dyes and Mix-n-Stain antibody labeling technology are covered by granted and pending U.S. and international patents.
We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to btinfo@biotium.com.

Antibody Labeling Kits

Antibody Labeling Kits

Dye	Ex/Em (nm)	SE labeling	Mix-n-Stain™ 5-20 ug	Mix-n-Stain™ 20-50 ug	Mix-n-Stain™ 50-100 ug	Mix-n-Stain™ Maxi 1 mg	Mix-n-Stain™ Nanobody 5-20 ug	Mix-n-Stain™ Nanobody 20-50 ug
CF®350	347/448	92210	92270	92250	92230	92420		
CF®405L	413/547	92228	92303	92304	92305			
CF®405M	416/452	92212	92272	92252	92232	92404		
CF®405S	404/431	92211	92271	92251	92231	92421	92502	92503
CF®430	426/498		92316	92317	92318			
CF®440	440/515		92319	92320	92321			
CF®450	450/538		92322	92323	92324			
CF®488A	490/515	92213	92273	92253	92233	92405	92504	92505
CF®514	516/548		92331	92332	92333			
CF®532	527/558	92208	92289	92290	92291			
CF®543	541/560	92209	92287	92267	92247			
CF®555	555/565	92214	92274	92254	92234	92406		
CF®568	562/583	92215	92275	92255	92235	92407	92506	92507
CF®570	568/591		92334	92335	92336			
CF®583	586/609		92337	92338	92339			
CF®594	593/614	92216	92276	92256	92236	92408	92508	92509
CF®633	630/650	92217	92277	92257	92237	92409		
CF®640R	642/662	92225	92278	92258	92245		92510	92511
CF®647	650/665	92218	92279	92259	92238	92410	92512	92513
CF®660C	667/685	92219	92280	92260	92239			
CF®660R	663/682	92223	92281	92261	92243			
CF®680	681/698	92220	92282	92262	92240	92422		
CF®680R	680/701	92226	92283	92263	92246		92514	92515
CF®700	695/720		92425	92426	92427			
CF®750	755/777	92221	92284	92264	92241	92423		
CF®770	770/797	92222	92285	92265	92242	92424		
CF®790	784/806		92288	92268	92248			
CF®800	797/816		92428	92429	92430			
CF®820	822/835		92431	92432	92433			

Labeling Kits for Other Conjugates

Conjugate	Ex/Em (nm)	SE labeling	Mix-n-Stain™ 5-20 ug	Mix-n-Stain™ 20-50 ug	Mix-n-Stain™ 25-50 ug	Mix-n-Stain™ 50-100 ug	Mix-n-Stain™ Maxi 1 mg	Mix-n-Stain™ Nanobody 5-20 ug	Mix-n-Stain™ Nanobody 20-50 ug
Biotin		92224	92286	92266		92244		92500	92501
FITC	494/518		92294	92295		92296	92411		
R-PE	496, 546, 565/578				92298	92299			
APC	650/660				92306	92307			
PerCP	482/677				92308	92309			
Cyanine 555	555/565		92412	92413		92414	92415		
Cyanine 647	650/665		92416	92417		92418	92419		
RPE-CF®647T	496/665				92340	92341	92346		
APC-CF®750T	650/780				92310	92311			

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Cy is a registered trademark of Cytiva.

Cell Viability Assays

Live-or-Dye™ Fixable Viability Stains

Live-or-Dye™ Fixable Viability Staining Kits are cell membrane-impermeant amine-reactive dyes designed for highly specific discrimination between live and dead cells during flow cytometry and microscopy. The staining is extremely stable, allowing cells to be fixed and permeabilized without loss of signal or dye transfer between cells.

Biotium offers a selection of 14 different Live-or-Dye™ viability stains spanning the fluorescence spectrum. (Fig. 1). Live-or-Dye NucFix™ Red is a unique, cell membrane-impermeant dye that specifically stains the nuclei of dead cells. NucFix™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

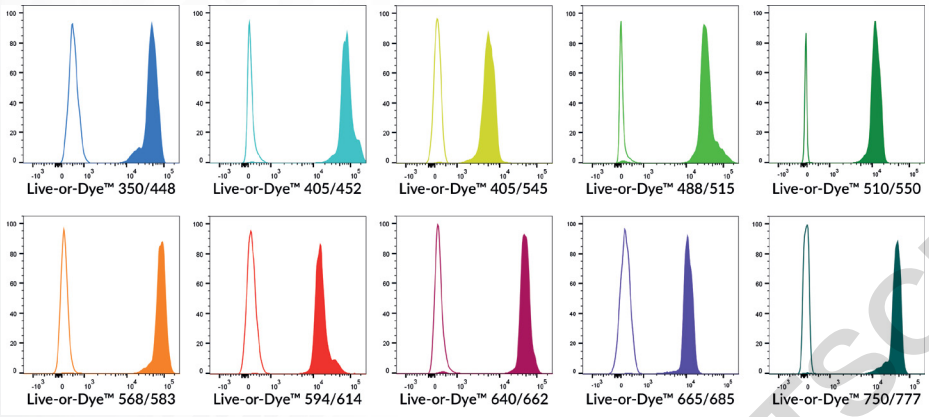


Figure 1. Jurkat cells were either left untreated or killed by heating to 56°C for 45 minutes, then stained with Live-or-Dye™ cell stain shown on each histogram x-axis. Heat killed cells (solid peaks) showed much higher fluorescence intensity compared to live cells (white peaks), allowing the two populations to be clearly distinguished.

Calcein AM Cell Viability Assay

Calcein AM is a non-fluorescent, membrane-permeant compound. Esterase activity in the cytoplasm of viable cells converts Calcein AM to the green fluorescent, membrane-impermeant compound calcein, which is retained in viable cells with intact plasma membranes (Fig. 2). The Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells pairs Calcein AM with the red fluorescent vital dye Ethidium Homodimer III for quantitation of live and dead cells.

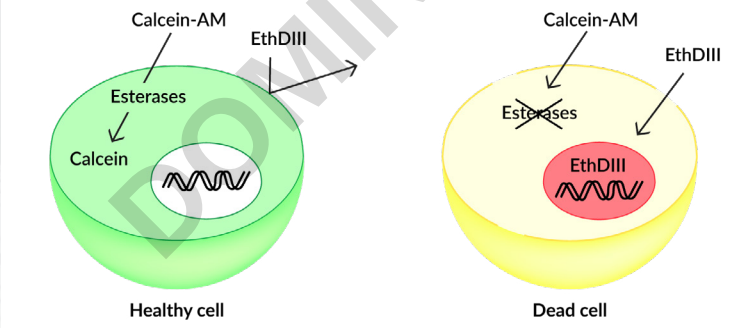


Figure 2. Principle of Calcein AM viability assay. Calcein AM is membrane-permeant and non-fluorescent. Upon entering a live cell, cellular esterases cleave it into calcein, a green fluorescent dye that is retained in the cell. Dead cells won't have active esterases and will therefore not fluoresce. Ethidium Homodimer III (EthD-III) is membrane-impermeant and stains dead cells with red fluorescence.

Live-or-Dye™ Advantages

- Offers the most available colors
- Dye options designed for spectral flow
- Highly dead cell selective
- Withstands fixation and permeabilization
- Stable in solution for at least a year
- Covalent labeling with reactive dyes

Live-or-Dye™ Kits

Product	Ex/Em (nm)	Cat. No. 50 Rxns	Cat. No. 200 Rxns
Live-or-Dye™ 350/448	347/448	32002-T	32002
Live-or-Dye™ 375/600*	375/595	32014-T	32014
Live-or-Dye™ 405/452	408/452	32003-T	32003
Live-or-Dye™ 405/545	395/545	32009-T	32009
Live-or-Dye™ 510/550*	516/549	32012-T	32012
Live-or-Dye™ 488/515	490/515	32004-T	32004
Live-or-Dye™ 568/583	562/583	32005-T	32005
Live-or-Dye™ 594/614	593/614	32006-T	32006
Live-or-Dye™ 615/740*	674/813	32015-T	32015
Live-or-Dye™ 640/662	642/662	32007-T	32007
Live-or-Dye™ 665/685*	667/685	32103-T	32013
Live-or-Dye™ 750/777	755/777	32008-T	32008
Live-or-Dye™ 787/808	783/803	32011-T	32011
Live-or-Dye NucFix™ Red	520/593**	32010-T	32010

* Spectrally unique dyes designed for spectral flow applications
** With DNA

Bacterial Viability Assays

Viability/Cytotoxicity Assay kit for Bacteria

In this kit, membrane permeable green fluorescent dye DMAO stains all bacteria, and Ethidium Homodimer III stains dead cells with red fluorescence. The kit is suitable for analysis by flow cytometry, fluorescence microscopy, or fluorescence microplate reader.

Bacterial Viability and Gram Stain Kit

CF®488A Wheat Germ Agglutinin stains gram-positive cells green, and Ethidium Homodimer III stains dead cells red. The also kit includes DAPI to stains all cells blue. This kit is suitable for analysis by flow cytometry, fluorescence microscopy, or fluorescence microplate reader.

Cell Viability Kits for Flow Cytometry

Cat. No.	Product	Unit Size
30002-T	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	150 assays
30002		300 assays
30026	Calcein AM Cell Viability Assay Kit	1000 assays
30027	Viability/Cytotoxicity Assay kit for Bacteria Live & Dead Cells	100-1000 assays
32001	Bacterial Viability and Gram Stain Kit	200 assays

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Cell Proliferation and Cell Cycle

ViaFluor® SE Cell Proliferation Kits

ViaFluor® SE Cell Proliferation Dyes diffuse passively into live cells and are used for long-term cell labeling. They are fluorogenic cell membrane permeant esters that are converted to reactive fluorescent dyes inside of cells by cytoplasmic esterases. The dyes then covalently react with amine groups on intracellular proteins, forming fluorescent conjugates that are retained in the cell. Each cell division that occurs after labeling is revealed by the appearance of a successively dimmer fluorescent peak on a flow cytometry histogram (Fig. 1). The dyes also can be used for microscopy imaging of cell morphology, or to track cells in co-cultures.

ViaFluor® CFSE is the classic cell proliferation dye, detected in the FITC channel. Biotium designed ViaFluor® 488 to be less a toxic, less leaky, and more fixable green dye than CFSE. We also offer blue fluorescent ViaFluor® 405 for the violet laser, with excellent performance comparable to CellTrace™ Violet at a lower cost.

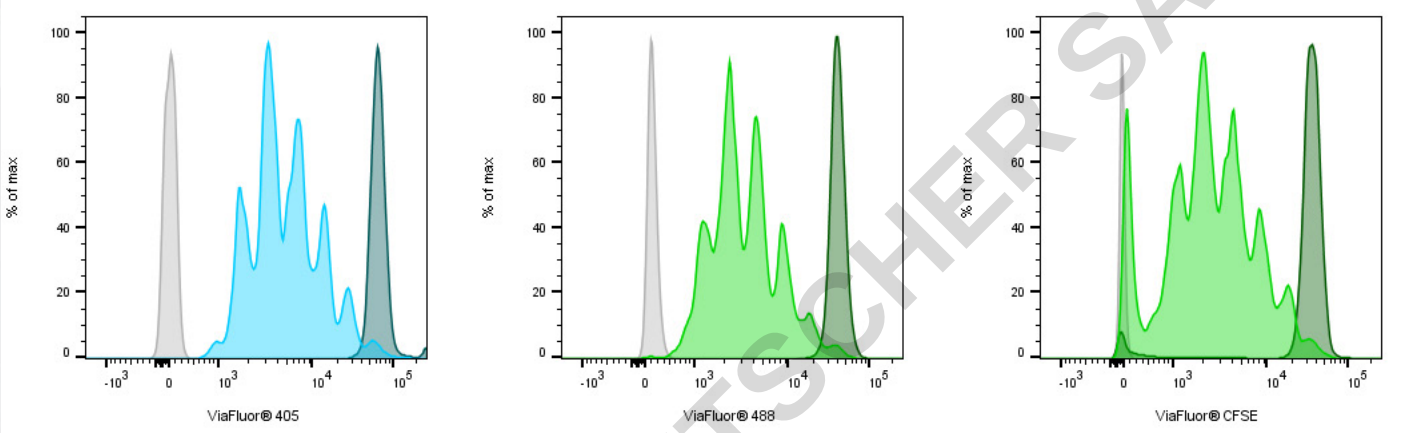


Figure 1. Human PBMCs were stained with ViaFluor® 405 (left), ViaFluor® 488 (center), or ViaFluor® CFSE (right). Cells were stimulated with Dynabeads® Human T-Activator CD3/CD28 beads and 100 ng/mL IL-2. Cells were analyzed 4 days post-induction. CD3+ T-cells are shown. Unstimulated cells (dark peaks) and unstained cells (gray) are shown for comparison.

ViaFluor® SE Cell Proliferation Kits

Cat. No.	Product	Ex/Em (nm)	Channel
30068	ViaFluor® 405 SE Cell Proliferation Kit	408/452	Pacific Blue®
30086	ViaFluor® 488 SE Cell Proliferation Kit	493/532	FITC
30050	ViaFluor® CFSE Cell Proliferation Kit	495/519	FITC

ViaFluor® 405 and ViaFluor® 488 SE Advantages

- For flow cytometry, microscopy, or live cell imaging systems
- Less toxic and better retained than CFSE
- ViaFluor® 488 SE has less bleed into the PE channel than CFSE
- Excellent peak separation
- Super bright for multi-generation tracking

Cell Cycle Analysis

RedDot™1 is a novel far-red nuclear stain developed at Biotium. RedDot™1 is a live cell stain similar to DRAQ®5 that can be used for cell cycle distribution analysis (Fig. 2). In addition, RedDot™1 cell cycle analysis does not require an RNase step unlike the classic nuclear stain Propidium Iodide (PI). RedDot™1 can also be used as a far-red nuclear counterstain for live cells in microscopy. 7-AAD and NucSpot® Far-Red can also be used for cell cycle analysis without an RNase step.

Dyes for Cell Cycle Analysis

Cat. No.	Product	Unit Size
40060-1	RedDot™1 Far-Red Nuclear Stain, 200X in Water	1 mL
40060		250 uL
40060-T		25 uL
40016	Propidium Iodide (PI)	100 mg
40017	Propidium Iodide (PI), 1 mg/mL in water	10 mL
40037	7-AAD	1 mg
40084		1 mL
40085	NucSpot® Far-Red, 1000X in DMSO	0.5 mL
40085-T		50 uL

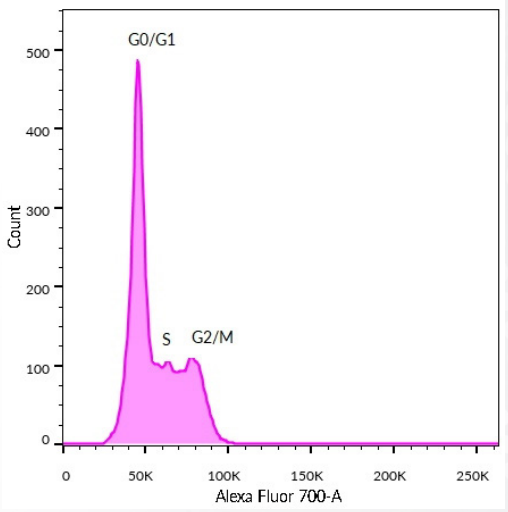


Figure 2. RedDot™1 staining for cell cycle distribution analysis. Live Jurkat cells were stained with 1X RedDot™1 for 30 minutes at 37°C, then analyzed using a BD LSRII flow cytometer with 633 nm excitation and 710/50 BP emission filter.

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RedDot1 is covered by pending U.S. and international patents. DRAQ5 is a trademark of Biostatus Limited.

NucView® Caspase-3 Substrates

Real-Time Apoptosis Detection in Living Cells

NucView® Caspase-3 substrates are novel fluorescent probes that allows real-time detection of caspase-3/7 activity in intact cells. In contrast to other fluorogenic caspase substrates or fluorescent caspase inhibitor based (FLICA) assays, NucView® substrates can be used to detect caspase-3/7 activity in cells without inhibiting apoptosis progression. NucView® is made by attaching a nucleic acid binding dye to the caspase-3/7 substrate peptide sequence DEVD. This uncleaved substrate is unable to bind to DNA and remains non-fluorescent. Once the substrate is cleaved by caspase-3/7 in apoptotic cells, it releases the high-affinity fluorescent DNA dye, which stains the cell nucleus with a bright and stable fluorescent signal (Fig. 1).

We offer green fluorogenic NucView® 488 Caspase-3 Substrate and kits which have been validated in more than a hundred published studies and cell types. Our NucView® 488 and MitoView™ 633 Apoptosis Kit may be used for dual detection of caspase activity and mitochondrial membrane potential (Fig. 2). We also offer dual apoptosis detection kits featuring NucView® 488 paired with red or far-red Annexin V conjugates. NucView® substrates are also available as blue fluorogenic NucView® 405 Caspase-3 Substrate using the 405 nm laser line, and as orange fluorogenic NucView® 530 Caspase-3 Substrate.

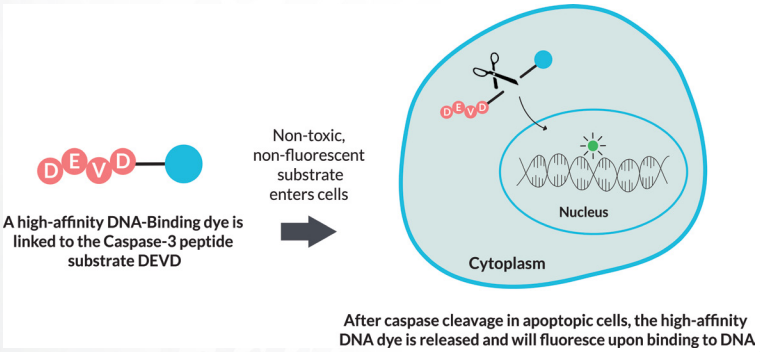


Figure 1. Principle of caspase-3/7 detection using NucView® caspase-3 substrates.

NucView® Advantages

- Rapid, no-wash, endpoint or real-time assays
- Non-toxic, allowing multi-day experiments
- For flow cytometry, microscopy, or live cell imaging systems
- Dual detection of caspase activity and nuclear morphology
- Formaldehyde-fixable
- Published in over 200 scientific papers and validated in more than 100 cell lines

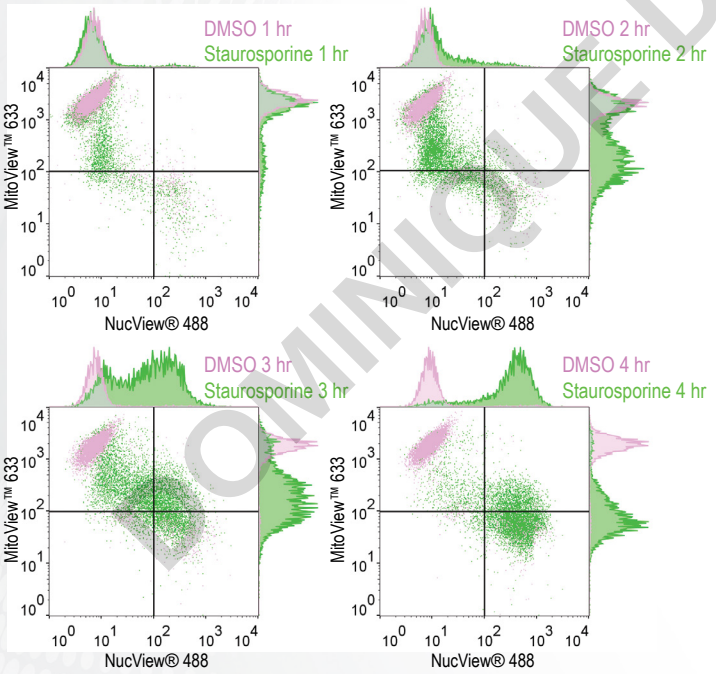


Figure 2. Flow cytometry analysis of Jurkat cells treated with staurosporine (green) to induce apoptosis, or DMSO controls (pink), using the NucView® 488 and MitoView™ 633 Apoptosis Kit. Fluorescence was analyzed on a BD FACSCalibur™ flow cytometer. As apoptosis progresses over time in staurosporine-treated cells, NucView® 488 signal (x-axis) increases and mitochondrial membrane potential measured by MitoView™ 633 staining (y-axis) decreases.

NucView® Substrates and Kits

Cat. No.	Product	Ex/Em (nm)	Unit Size
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO	500/530	100 uL
10403	NucView® 488 Caspase-3 Substrate, 1 mM in PBS		100 uL
30029	NucView® 488 Caspase-3 Assay Kit for Live Cells		100 assays
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO	429/469	100 uL
10407	NucView® 405 Caspase-3 Substrate, 1 mM in PBS		100 uL
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO	528/563	100 uL
10408	NucView® 530 Caspase-3 Substrate, 1 mM in PBS		100 uL
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit	500/530, 622/648	100 assays
30072	NucView® 488 and RedDot™2 Apoptosis & Necrosis Kit	500/530, 665/695	100 assays
30067	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V	500/530, 593/614	50 assays
30073	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®640R Annexin V	500/530, 642/662	50 assays
30030	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and Texas Red®-Annexin V	500/530, 594/615	50 assays

NucView enzyme substrate technology is covered by granted US and/or international patents. **We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to btinfo@biotium.com.**

FACSCalibur is a trademark of BD Biosciences.

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Apoptosis and Necrosis Assays

Annexin V Conjugates

Annexin V is a 35-36 kDa protein with high affinity for phosphatidylserine (PS). During apoptosis, PS is translocated from the inner to the outer leaflet of the plasma membrane, where it can be stained by fluorescent conjugates of Annexin V, for detection of apoptotic cells by flow cytometry (Fig. 1) or fluorescence microscopy. Biotium offers Annexin V conjugates and kits featuring our exceptionally bright and photostable CF® Dyes. For example, our CF®488A green fluorescent Annexin V conjugate is much brighter and more photostable than the traditional FITC-Annexin V, allowing the use of 10-fold less conjugate in staining. Our azide-free lyophilized Annexin V conjugates are suitable for no-wash, real-time cell staining or *in vivo* imaging.

Annexin V Conjugates

Cat. No.	Conjugate	Ex/Em (nm)	Unit Size
29012	Annexin V, CF®350, 50 ug/mL	347/448	0.5 mL
29012R-5ug	Annexin V, CF®350, Azide-Free, Lyophilized		5 ug
29009	Annexin V, CF®405M, 50 ug/mL	408/452	0.5 mL
29009-5ug	Annexin V, CF®405M, Azide-Free, Lyophilized		5 ug
29083	Annexin V, CF®450, 50 ug/mL	450/538	0.5 mL
29083R-5ug	Annexin V, CF®450, Azide-Free, Lyophilized		5 ug
29005	Annexin V, CF®488A, 50 ug/mL	490/515	0.5 mL
29005R-5ug	Annexin V, CF®488A, Azide-Free, Lyophilized		5 ug
29004	Annexin V, CF®555, 50 ug/mL	555/565	0.5 mL
29004R-5ug	Annexin V, CF®555, Azide-Free, Lyophilized		5 ug
29010	Annexin V, CF®568, 50 ug/mL	562/583	0.5 mL
29010R-5ug	Annexin V, CF®568, Azide-Free, Lyophilized		5 ug
29085	Annexin V, CF®583R, 50 ug/mL	586/609	500 uL
29085R-5ug	Annexin V, CF®583R, Azide-Free, Lyophilized		5 ug
29011	Annexin V, CF®594, 50 ug/mL	593/614	0.5 mL
29011R-5ug	Annexin V, CF®594, Azide-Free, Lyophilized		5 ug
29008	Annexin V, CF®633, 50 ug/mL	630/650	0.5 mL
29008R-5ug	Annexin V, CF®633, Azide-Free, Lyophilized		5 ug
29014	Annexin V, CF®640R, 50 ug/mL	642/662	0.5 mL
29014R-5ug	Annexin V, CF®640R, Azide-Free, Lyophilized		5 ug
29003	Annexin V, CF®647, 50 ug/mL	650/665	0.5 mL
29003R-5ug	Annexin V, CF®647, Azide-Free, Lyophilized		5 ug
29069	Annexin V, CF®660R, 50 ug/mL	663/682	0.5 mL
29069R-5ug	Annexin V, CF®660R, Azide-Free, Lyophilized		5 ug
29007	Annexin V, CF®680, Azide-Free, Lyophilized	681/698	25 ug
29070	Annexin V, CF®680R, Azide-Free, Lyophilized	680/701	25 ug
29082	Annexin V, CF®700, Azide-Free, Lyophilized	699/737	25 ug
29006	Annexin V, CF®750, Azide-Free, Lyophilized	755/777	25 ug
29046	Annexin V, CF®770, Azide-Free, Lyophilized	770/797	25 ug
29047	Annexin V, CF®790, Azide-Free, Lyophilized	784/806	25 ug
29078	Annexin V, CF®800, Azide-Free, Lyophilized	797/816	25 ug
29001	Annexin V, FITC, 50 ug/mL	490/525	0.5 mL
29045-100 uL	Annexin V, R-PE	496, 546, 565/578	20 assays
29045-500 uL			100 assays
29057-100 uL	Annexin V, APC	633, 640/660	20 assays
29057-500 uL			100 assays
29013	Annexin V, Biotin, 50 ug/mL	N/A	0.5 mL
99902	5X Annexin V Binding Buffer	N/A	15 mL

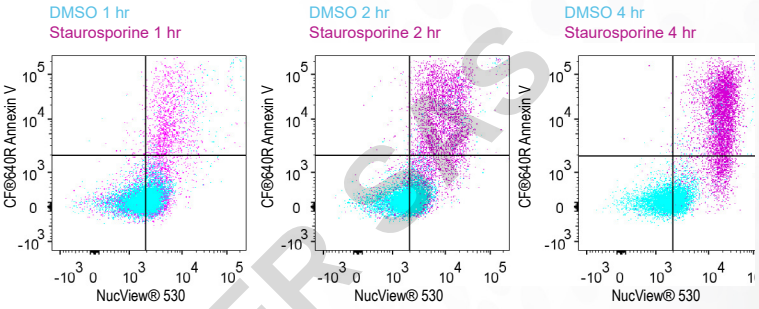


Figure 1. Flow cytometry analysis of Jurkat cells treated with staurosporine (pink) to induce apoptosis, or DMSO as a control (blue), using NucView® 530 Caspase-3 Substrate and CF®640R Annexin V. Fluorescence was analyzed on a BD LSRII flow cytometer. As apoptosis progresses over time in staurosporine-treated cells, both NucView® 530 signal (x-axis) and CF®640R Annexin V staining (y-axis) increases.

CF®488A Annexin V Apoptosis Kits with PI or 7-AAD

Biotium offers kits that include CF®488 Annexin V paired with the red fluorescent nuclear stain Propidium Iodide (PI) or far-red fluorescent 7-AAD. These kits offer rapid staining for simultaneous detection of late apoptotic and necrotic cells for flow cytometry.

Apoptosis & Necrosis Quantitation Kits

This kit contains CF®488A Annexin V and the dead cell stain Ethidium Homodimer III (a novel membrane-impermeant nucleic acid dye developed at Biotium with higher affinity for DNA and higher fluorescence quantum yield than Propidium Iodide). The Apoptotic, Necrotic, and Healthy Cells Quantitation Kit also includes includes Hoechst 33342, a blue fluorescent DNA dye for visualizing healthy cells.

Dual Apoptosis Assay Kits

Annexin V conjugated to our deep-red CF®594 Dye or far-red CF®640R Dye is offered together with NucView® 488 Caspase-3 Substrate for simultaneous detection of caspase-3 activity and phosphatidylserine exposure by fluorescence microscopy or flow cytometry (see page 8 for more information on NucView® substrates).

Apoptosis and Necrosis Detection Kits

Cat. No.	Product	Unit Size
30065	Apoptosis & Necrosis Quantitation Kit Plus	50 assays
30066	Apoptotic, Necrotic, & Healthy Cells Quantitation Kit Plus	50 assays
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit	100 assays
30061	CF®488A Annexin V and PI Apoptosis Kit	100 assays
30067	Dual Apoptosis Assay with NucView® 488 and CF®594 Annexin V	50 assays
30072	NucView® 488 & RedDot™ 2 Apoptosis & Necrosis Kit	100 assays
30073	Dual Apoptosis Assay with NucView® 488 and CF®640R Annexin V	50 assays

DOMINIQUE DUTSCHER SAS

Membrane Potential Dyes and Other Accessories

MitoView™ Dyes

MitoView™ dyes are fluorogenic mitochondrial stains for live cells. The dyes rapidly stain mitochondria without a wash step, and are non-toxic for live cell imaging. They are available with blue, green, far-red, and near-infrared fluorescence. MitoView™ 633 can be used to monitor mitochondrial membrane potential by microscopy or flow cytometry (Fig. 1). We also offer MitoView™ Green, a potential-independent mitochondrial dye that can be detected following mitochondrial depolarization, or after fixation. MitoView™ dyes stain mitochondria in yeast, and also stain bacteria (gram-positive and gram-negative).

MitoView™ Dyes

Cat. No.	Product	Ex/Em (nm)	Potential-dependent?
70070	MitoView™ 405	398/440	Partial[3]
70054	MitoView™ Green	490/523	No
70055	MitoView™ 633	622/648[1]	Yes
70075	MitoView™ 650	644/670	Partial[3]
70068	MitoView™ 720	720/758[2]	Partial[3]

[1] MitoView™ 633 also has red fluorescence in the Cy®3/rhodamine channel. It is not recommended for imaging with other red fluorescent probes.
[2] While optimal for Cy®7 settings, MitoView™ 720 is bright enough to be imaged in the Cy®5 channel, and can be combined with visible red fluorescent probes.
[3] Dyes with partial mitochondrial membrane potential dependence localize to the cytoplasm after mitochondrial depolarization, but still retain fluorescence.

Other Mitochondrial Dyes and Kits

We offer a wide selection of classic mitochondrial membrane-potential dyes suitable for flow cytometry. Our JC-1 Mitochondrial Membrane Potential Detection Kit allows ratiometric detection of mitochondrial membrane potential using JC-1 dye. The kit includes a step-by step protocol and ready-to-use reagents for flow cytometry or fluorescence microplate reader.

We also offer JC-1 iodide and chloride salts, Rhodamine 123, and TMRE/TMRM. For fixed cells and tissues, we recommend using mitochondrial localized antibodies. We offer several mitochondrial localized primary antibodies conjugated to our bright and photostable CF® Dyes.

Visit our website at www.biotium.com to view our full selection of mitochondrial dyes and antibodies.

Other Mitochondrial Dyes and Kits

Cat. No.	Product	Ex/Em (nm)	Potential-dependent?
30001	JC-1 Mitochondrial Membrane Potential Detection Kit	510/527; 585/590*	Ratiometric*
70014	JC-1, Iodide Salt		
70011	JC-1, Chloride Salt		
70016	TMRE	549/574	Yes
70005	TMRE, 2 mM in DMSO		
70017	TMRM	548/573	Yes
70010	Rhodamine 123	505/534	Yes

*JC-1 forms red fluorescent aggregates in polarized mitochondria, and green fluorescent monomers in cytoplasm

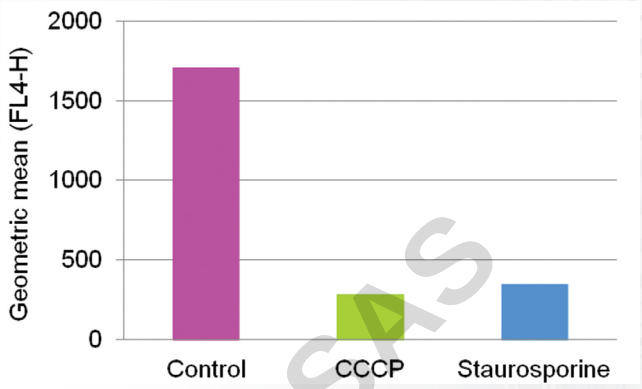


Figure 1. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

Flow Cytometry Accessories

The Flow Cytometry Fixation/Permeabilization Kit contains optimally formulated buffers for fixation and permeabilization of suspension cells for immunostaining of intracellular antigens for analysis by flow cytometry. Fixation, permeabilization, and permeabilization/blocking buffers also are available separately. Biotium also offers an array of flow cytometry accessory products including PBS buffer, mini cell scrapers, and blocking reagents.



Figure 2. Flow Cytometry Fixation/Permeabilization Kit.

Flow Cytometry Accessories

Cat. No.	Product	Unit Size
23006	Flow Cytometry Fixation/Permeabilization Kit	50 tests
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer (5X)	100 mL
22020	10X Phosphate-Buffered Saline (PBS)	4 L
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22013	Bovine Serum Albumin, Fraction V	50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22002	Tween®-20	50 mL
22003	Mini Cell Scrapers	Pack of 200

TWEEN is a registered trademark of Uniqema Americas LLC; Cy is a registered trademark of Cytiva.

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Spectral Flow Cytometry

Spectral flow cytometry is a rapidly growing technology with significantly enhanced multiplexing capabilities over conventional flow cytometry. Where conventional flow cytometer instruments can detect panels with more than a dozen fluorophores, spectral flow cytometers can accommodate panels with upwards of 40 dyes.

Spectrally Unique Dyes Only At Biotium

Biotium is proud to develop spectrally unique dyes aimed at filling emission gaps between available fluorophores, allowing users to maximize the multiplexing advantages of spectral flow cytometry. Our CF® Dye product line boasts the largest selection of spectrally unique fluorophores, many of which are licensed by Cytek® Biosciences for use in spectral flow panels. See Fig. 1 for a similarity matrix of CF® Dyes that have been validated in spectral flow by Cytek®. With our Mix-n-Stain CF® Dye Antibody Labeling Kits, these dyes can be easily conjugated to your antibodies.

In addition, several dyes from our line of Live-or-Dye™ Fixable Viability Staining Kits were designed specifically for spectral flow: Live-or-Dye™ 510/550, 665/685, 375/600, and 615/740 (see page 6 for more information on Live-or-Dye™ Fixable Viability Staining Kits).

Similarity Index of CF® Dyes and Other Labels

	CF®405L	CF®405M	CF®430	CF®450	CF®488A	CF®514	CF®532	CF®543	CF®555	CF®568	CF®570	CF®583	CF®594	CF®633	CF®640R	CF®647	CF®660C	CF®660R	CF®680	CF®680R	CF®700	CF®750	CF®770	APC	PE	PerCP	Cy®3
CF®405L	1																										
CF®405M	0.13	1																									
CF®430	0.7	0.52	1																								
CF®450	0.53	0.12	0.51	1																							
CF®488A	0.01	0	0.02	0.78	1																						
CF®514	0.34	0.04	0.24	0.78	0.57	1																					
CF®532	0.5	0.04	0.29	0.69	0.34	0.89	1																				
CF®543	0.19	0.01	0.09	0.17	0.05	0.21	0.47	1																			
CF®555	0	0	0	0.05	0.02	0.09	0.3	0.96	1																		
CF®568	0.06	0	0.03	0.05	0.01	0.05	0.27	0.95	0.97	1																	
CF®570	0.02	0	0.01	0.04	0.01	0.06	0.27	0.9	0.95	0.98	1																
CF®583	0.07	0	0.02	0.04	0.01	0.05	0.2	0.56	0.64	0.72	0.84	1															
CF®594	0.06	0	0.02	0.04	0	0.03	0.14	0.4	0.5	0.52	0.65	0.9	1														
CF®633	0.02	0	0.01	0.01	0	0.01	0.02	0.03	0.05	0.05	0.09	0.15	0.16	1													
CF®640R	0.01	0	0	0	0	0	0.01	0.02	0.03	0.03	0.05	0.08	0.09	0.98	1												
CF®647	0	0	0	0	0	0	0	0.01	0.02	0.02	0.04	0.06	0.08	0.95	0.98	1											
CF®660C	0.01	0	0	0	0	0	0	0.01	0.02	0.02	0.03	0.05	0.07	0.85	0.86	0.92	1										
CF®660R	0.01	0	0	0	0	0	0.01	0.01	0.02	0.02	0.05	0.07	0.1	0.86	0.87	0.92	0.99	1									
CF®680	0.01	0	0	0	0	0	0	0.01	0.01	0.01	0.02	0.03	0.05	0.74	0.7	0.74	0.91	0.91	1								
CF®680R	0.01	0	0	0.01	0	0.01	0.01	0.01	0.02	0.02	0.04	0.06	0.08	0.73	0.68	0.72	0.88	0.89	0.99	1							
CF®700	0.01	0	0	0	0	0	0	0	0.01	0.01	0.01	0.02	0.03	0.6	0.54	0.53	0.66	0.67	0.86	0.88	1						
CF®750	0.01	0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.2	0.2	0.15	0.23	0.3	0.31	0.34	0.44	1					
CF®770	0.01	0	0	0.01	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.16	0.15	0.12	0.18	0.25	0.26	0.3	0.38	0.95	1				
APC	0.03	0	0.01	0.01	0	0.01	0.02	0.05	0.07	0.08	0.13	0.21	0.23	0.97	0.94	0.9	0.75	0.77	0.6	0.6	0.48	0.15	0.11	1			
PE	0.16	0.01	0.07	0.19	0.05	0.24	0.53	0.97	0.91	0.88	0.83	0.49	0.33	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0.04	1		
PerCP	0.03	0.01	0.01	0.03	0.01	0.06	0.07	0.05	0.08	0.07	0.14	0.22	0.26	0.27	0.23	0.23	0.21	0.25	0.15	0.19	0.09	0.04	0.03	0.34	0.06	1	
Cy®3	0.04	0.04	0.05	0.1	0.04	0.13	0.34	0.95	0.99	0.96	0.96	0.7	0.56	0.1	0.06	0.06	0.05	0.06	0.03	0.05	0.02	0.01	0.01	0.14	0.91	0.18	1

Figure 1. The Similarity Index is a measure of spectral similarity on a scale from 0 to 1. Values close to 0 indicate that the full spectrum signatures of the two dyes are very different from each other, and values close to 1 indicate that the signatures are very similar to each other. When designing multiplex panels, it is important to choose unique fluorophores with minimal spectral similarity. Data was provided by Cytek® Biosciences from CD4 labeling of human PBMCs using a Cytek® Aurora flow cytometer.

CF® Dye Advantages

- Widest selection of spectrally unique dyes to accomodate highly complex panels
- Greatest coverage across visible and near-IR wavelengths
- Superior brightness and signal-to-noise over alternative fluorophores

Still searching for the right dye?

If you are looking for a spectrally unique dye not listed on our website, please contact **techsupport@biotium.com**. We may be able to develop a new dye to fit your needs.

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About Us

At Biotium, we are dedicated to developing cutting-edge fluorescent solutions for life science research. Our efforts have resulted in a growing number of unique and industry-leading fluorescence-based technologies for a wide array of biological research applications. Our products are available in the U.S. through our website, and worldwide through our extensive network of domestic and international distributors.

We license our technologies to a number of international biotechnology companies, and collaborate with academic laboratories to develop tools for the constantly evolving research community. We welcome inquiries about licensing the use of our dyes, technologies, or trademarks; email us at btinfo@biotium.com.

Biotium implements a Quality System, certified by QAS according to Standard QAS ISO 9001:2015.

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