



Products for Flow Cytometry

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Flow Cytometry Laser Lines for CF™ Dyes

Visible spectrum

Far-red

Near-infrared

Optimal laser line	CF™ dye	λ_{Ex} (nm)	λ_{Em} (nm)	Replacement for	Advantages
Mercury arc 366, 405, 435 nm He-Cd 325 nm 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
Mercury arc 366, 405, 435 nm 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	408	452	BD Horizon™ V450, eFluor® 450, Pacific Blue®	- More photostable than Pacific Blue® dye - Less spill-over in the 525/50 green channel - Highly water-soluble
	CF™405L	395	545	Pacific Orange®	Long stokes shift allows multicolor detection from violet laser excitation
Argon 488 nm	CF™488A	490	515	ATTO 488, Alexa Fluor® 488, Cy®2, DyLight® 488, FAM, FITC, Fluorescein	- Yields biologically 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™514	516	548	AlexaFluor® 514	- Green fluorophore that can be distinguished from CF™488A by spectral unmixing - Extremely photostable - Highly water-soluble and pH-insensitive
Argon 488 nm Green He-Ne 532 nm	CF™532	527	558	AlexaFluor® 532, ATTO 532	- Significantly brighter than Alexa Fluor® 532 - Highly water-soluble and pH-insensitive
	CF™543	541	560	Alexa Fluor® 546, Tetramethylrhodamine (TAMRA)	- Significantly brighter than Alexa Fluor® 546 - Highly water-soluble and pH-insensitive
Argon 488 nm Green He-Ne 532 nm Yellow-Green 561 nm	CF™555	555	565	Alexa Fluor® 555, ATTO 550, Cy®3, DyLight® 549, TRITC	- Brighter than Cy®3 - Comparable to Alexa Fluor® 555
Yellow-Green 561 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™594	593	614	Alexa Fluor® 594, ATTO 594, DyLight® 594, Texas Red®	- Yields the brightest conjugates among spectrally similar dyes - Extremely photostable
	CF™620R	617	639	LightCycler® Red 640	- Highly fluorescent - Extremely photostable and highly water-soluble
He-Ne 633 nm	CF™633	630	650	Alexa Fluor® 633, Alexa Fluor® 647, Cy®5, DyLight® 633	- Yields the brightest antibody conjugates among spectrally similar dyes when excited at 633 nm or 635 nm - Far more photostable than Alexa Fluor® 647 - Highly water-soluble
	CF™640R	642	662	Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649	- Has the best photostability among dyes with Cy®5-like spectra - Yields highly fluorescent protein conjugates - Highly water-soluble and pH-insensitive
	CF™647	650	665	Alexa Fluor® 647, ATTO 647N, Cy®5, DyLight® 649	- Brighter than Cy®5 - Comparable to Alexa Fluor® 647 - 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
He-Ne 633 nm	CF™750	755	777	Alexa Fluor® 750, Cy®7, DyLight® 750, APC-Alexa Fluor® 750, IRDye® 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 - Compatible with LI-COR Odyssey® System
	CF™790	784	806	Alexa Fluor® 790	- Exceptionally bright and stable - Highly water soluble without bearing excessive charge

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

Biotium offers secondary antibodies and biomolecules conjugated to a wide selection of CF™ dyes. We also sell antibodies conjugated to R-phycoerythrin (R-PE), Allophycocyanin (APC) and tandem dyes. Visit www.biotium.com for a full listing of fluorescent conjugates for flow cytometry.

Biotium regularly adds new dye conjugate products to our catalog according to customer demand. If you are looking for a product not listed on our website, please let us know. We may be able to add it as a new product, or perform a custom conjugation for you.

Secondary Antibodies, Whole IgG
Chicken Anti-Goat
Chicken Anti-Mouse
Chicken Anti-Rabbit
Goat Anti-Guinea Pig
Goat Anti-Mouse
Goat Anti-Rabbit
Goat Anti-Swine
Rabbit Anti-Chicken
Rabbit Anti-Goat
Rabbit Anti-Guinea Pig

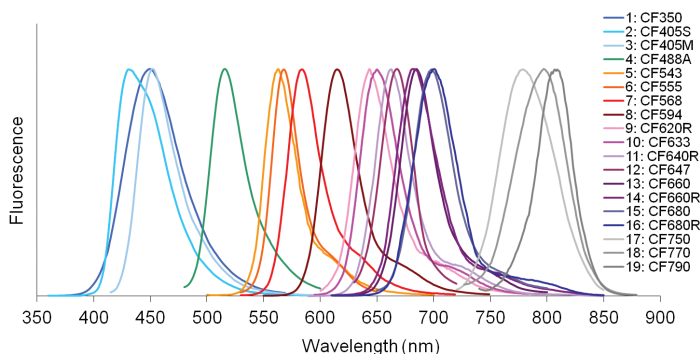
Secondary Antibodies, F(ab') ₂ fragments
Goat Anti-Mouse
Goat Anti-Rabbit

Isotype-Specific Secondary Antibodies
Goat Anti-Mouse IgG1
Goat Anti-Mouse IgG2a
Goat Anti-Mouse IgG2b
Goat Anti-Human IgG (Fc gamma)
Goat Anti-Human IgM (mu chain)
Goat Anti-Human IgA (alpha chain)

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
Rabbit Anti-Myc tag
Rabbit Anti-V5 tag
Rabbit Anti-FLAG tag
Mouse Monoclonal Anti-Biotin
Mouse Monoclonal Anti-Fluorescein

Secondary Antibodies, Highly Cross-Adsorbed for Multiple Labeling	Min X React
Bovine Anti-Goat	Bv, Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm
Donkey Anti-Chicken	Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm
Donkey Anti-Goat	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm
Donkey Anti-Guinea Pig	Bv, Ch, Gt, Hs, Hu, Ms, Rb, Sh, SHm
Donkey Anti-Human	Bv, Ch, GP, Gt, Hs, Ms, Rb, Rt, Sh, SHm
Donkey Anti-Mouse	Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm
Donkey Anti-Rabbit	Bv, Ch, Gt, GP, Hs, Hu, Ms, Sh, SHm
Donkey Anti-Rat	Bv, Ch, GP, Gt, Hs, Hu, Ms, Rb, Sh, SHm
Donkey Anti-Sheep	Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm
Goat Anti-Chicken	Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm
Goat Anti-Human	Bv, Hs, Ms
Goat Anti-Mouse	Bv, Hs, Hu, Rb, Sw
Goat Anti-Mouse (min X Rat)	Bv, Ch, Gt, GP Hs Hu Rb Rt, Sh, SHm
Goat Anti-Rabbit	Hu, Ms, Rt
Goat Anti-Rat	Bv, Hs, Hu, Rb
Rabbit Anti-Human	Ms
Rabbit Anti-Mouse	Hu
Rabbit Anti-Rat	Hu
Rabbit Anti-Sheep	Hu

Other Bioconjugates	Application
Annexin V	Apoptosis (phosphatidylserine) detection (see p. 7)
α-Bungarotoxin	Acetylcholine receptor probe
Bovine serum albumin	Fluid phase endocytosis tracer
Cholera Toxin Subunit B	GM1 receptor probe; lipid raft staining, endocytic vesicle tracking
Concanavalin A	Carbohydrate probe (lectin)
dNTPs	DNA probes, TUNEL assay
Phalloidin	Filamentous actin probe
Streptavidin	Detection of biotinylated probes
Transferrin (human)	Recycling endosome tracer
Wheat germ agglutinin	Carbohydrate probe (lectin), bacterial gram stain



Mix-n-Stain™ Antibody Labeling Kits

- The simplest antibody labeling protocol available
- Covalently label your antibody in 30 minutes
- No clean up of free dye required
- Tolerates common antibody buffer components
- Label antibody in the presence of excess stabilizer protein using the modified Mix-n-Stain™ protocol
- Choose between bright and photostable CF™ dyes, R-PE, APC or Per-CP.

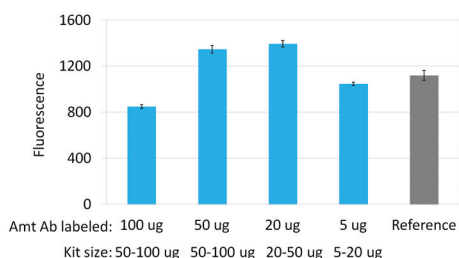


Figure 1. Flow cytometry analysis of Jurkat cells stained with CF™633 Mix-n-Stain labeled mouse anti-human CD3 antibodies (BD cat# 555330). For reference, cells were stained with commercial Alexa Fluor® 647 mouse anti-human CD3 (BD cat# 557706). Fluorescence was analyzed on a BD FACSCalibur flow cytometer in the FL4 channel.

Ordering Information

Reaction size*	Catalog numbers for phycobiliprotein Mix-n-Stain™ Kits		
	R-PE	APC	Per-CP
25-50 ug	92298	92306	92308
50-100 ug	92299	92307	92309

	Catalog numbers for CF™ Dye Mix-n-Stain™ Kits																			Other Mix-n-Stain™ Kits		
Reaction size*	CF350	CF405S	CF405L	CF405M	CF488A	CF532	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770	CF790	Biotin	FITC
5-20 ug	92270	92271	92303	92272	92273	92289	92287	92274	92275	92276	92277	92278	92279	92280	92281	92282	92283	92284	92285	92288	92286	92294
20-50 ug	92250	92251	92304	92252	92253	92290	92267	92254	92255	92256	92257	92258	92259	92260	92261	92262	92263	92264	92265	92268	92266	92295
50-100 ug	92230	92231	92305	92232	92233	92291	92247	92234	92235	92236	92237	92245	92238	92239	92243	92240	92246	92241	92242	92248	92244	92296

*One labeling reaction per kit.

Lightning Link® is registered trademark of Innova Biosciences. DyLight® is a registered trademark of Thermo Fisher Scientific.

CF™ Dye SE Protein Labeling Kits

SE protein labeling kits include everything you need to conjugate and purify up to 1 mg of antibody or other protein:

- 3 vials of lyophilized CF™ dye succinimidyl ester, sufficient for labeling 1 mg antibody each
- All required solvents, buffers and vials
- Ultrafiltration vials for quick and easy purification of antibody after labeling using a microcentrifuge
- Detailed protocols for conjugation, antibody purification, and determination of antibody concentration and degree of labeling (DOL)

Ordering Information

Catalog numbers for SE Protein Labeling Kits																			
CF350	CF405S	CF405M	CF405L	CF488A	CF532	CF543	CF555	CF568	CF594	CF633	CF640R	CF647	CF660C	CF660R	CF680	CF680R	CF750	CF770	biotin
92210	92211	92212	92228	92213	92208	92209	92214	92215	92216	92217	92225	92218	92219	92223	92220	92226	92221	92222	92224

Mix-n-Stain™ antibody labeling kits dramatically simplify the process of preparing fluorescently-labeled antibodies. Simply mix your antibody with the reaction buffer and dye provided in the kit. After 30 minutes of incubation, and without a separation step, you will have an antibody conjugate of the dye of your choice that is comparable to commercially-available fluorescent antibody conjugates (Figure 1).

Mix-n-Stain™ kits feature Biotium's CF™ dyes, which have advantages of brightness and photostability compared to other fluorescent dyes, such as the DyLight® dyes used in Lightning Link® antibody labeling kits (Figure 2). Mix-n-Stain™ kits for labeling antibodies with R-PE, APC, Per-CP, biotin, FITC and HRP are also available.

Mix-n-Stain™ labeling kits are available in several sizes for labeling different amounts of antibody (see ordering information below). Visit www.biotium.com to find the Mix-n-Stain™ kit that is right for you and to download the Mix-n-Stain™ product information sheet and flyer.

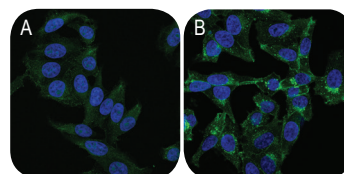


Figure 2. Mouse anti-transferrin receptor antibody (BD Biosciences) was labeled using Lightning Link® Rapid DyLight® 488 Conjugation Kit from Novus Biologicals (A) or Mix-n-Stain CF488A Antibody Labeling Kit (B). The CF™488A conjugate staining shows higher signal and more specific staining compared the DyLight® 488 conjugate.

Live-or-Dye™ Fixable Viability Stains

Live-or-Dye™ Fixable Viability Staining Kits are designed for discrimination between live and dead cells during flow cytometry and microscopy. Live-or-Dye™ Fixable Viability Stains are cell membrane impermeable amine-reactive dyes.

The dyes enter dead cells that have compromised membrane integrity and covalently label free amines on intracellular proteins.

Live-or-Dye™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells. The staining protocol has been optimized to maximize live/dead discrimination with minimal live cell staining, in order to prevent interference with immunostaining. Biotium offers a selection of eight different Live-or-Dye™ viability stains spanning the fluorescence spectrum, for maximal flexibility in multi-color analysis (see Figure 3 and the chart below).

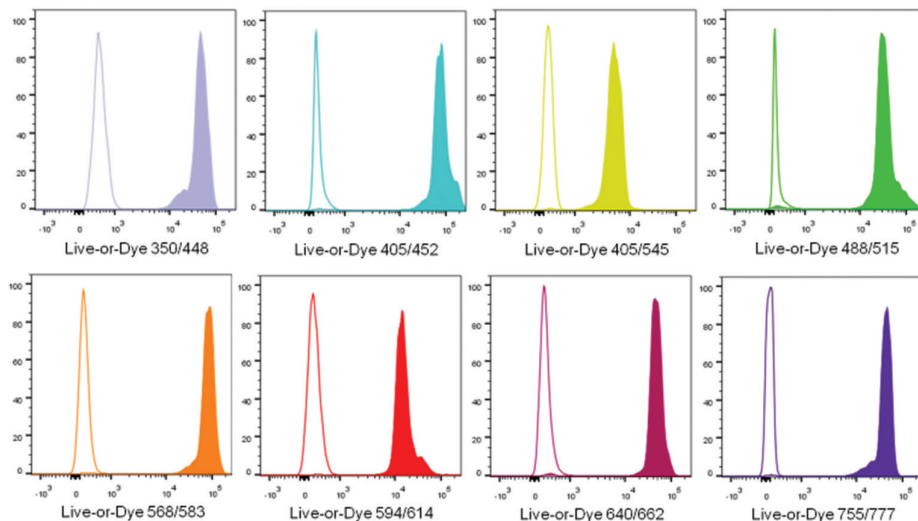


Figure 3. Jurkat cells were either left untreated or killed by heating to 56°C for 45 minutes, then stained according to the product protocol with the Live-or-Dye™ cell stain shown on each histogram x-axis. Heat killed cells (solid peaks) showed much higher fluorescence intensity compared to live cells (white peaks), allowing the two populations to be clearly distinguished. Results are shown for unfixed cells; nearly identical histograms were observed after cell fixation with 2% formaldehyde in PBS for 20 minutes at room temperature, followed by permeabilization with 0.1% Triton X-100 in PBS for 30 minutes at room temperature.

Ordering Information

Kit size*	Catalog numbers for Live-or-Dye™ Fixable Viability Staining Kits							
	Live-or-Dye™ 350/448	Live-or-Dye™ 405/452	Live-or-Dye™ 405/545	Live-or-Dye™ 488/515	Live-or-Dye™ 568/583	Live-or-Dye™ 594/614	Live-or-Dye™ 640/662	Live-or-Dye™ 750/777
50 reactions	32002-T	32003-T	32009-T	32004-T	32005-T	32006-T	32007-T	32008-T
200 reactions	32002	32003	32009	32004	32005	32006	32007	32008

Calcein-AM Cell Viability Assay

Calcein-AM is a non-fluorescent, membrane permeable 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 (see Figure 4). 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.

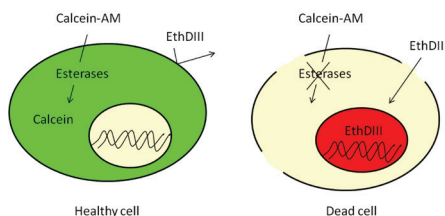


Figure 4. Principle of Calcein-AM viability assay. Calcein-AM is membrane-permeable and non-fluorescent. If it enters a live cell, cellular esterases cleave it into calcein, a green fluorescent dye that is retained in the cell. Dead cells won't have active esterases and thus won't become green fluorescent.

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. 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 kit includes DAPI to stains all cells blue. For analysis by flow cytometry, fluorescence microscopy, or fluorescence microplate reader.

Ordering Information

Catalog No.	Product Description	Unit Size
30026	Calcein AM Cell Viability Assay Kit	1000 assays
30002-T	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	150 assays
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	300 assays
30027	Viability/Cytotoxicity Assay kit for Bacteria Live & Dead Cells	100-1000 assays
32001	Bacterial Viability and Gram Stain Kit	1 kit (200 assays)

ViaFluor™ Cell Proliferation Kits

ViaFluor™ cell proliferation kits work exactly like the common cell proliferation dye CFSE but use Biotium's superior dye technology. ViaFluor™ kits contain dyes that diffuse passively into cells and covalently label intracellular proteins, resulting in long term cell labeling. They are non-fluorescent until they are hydrolyzed by intracellular esterases. The dyes then react with intracellular amines forming fluorescent conjugates that are retained in the cell. Immediately after staining, a single, bright fluorescent population will be detected by flow cytometry. With each cell division, daughter cells inherit roughly half of the fluorescent label, allowing the number of cell divisions that occur after labeling to be detected by the appearance of successively dimmer fluorescent peaks compared to undivided cells (Figure 5). ViaFluor™ staining is formaldehyde fixable. Each ViaFluor™ Cell Proliferation Assay Kit contains ten single use dye vials, anhydrous DMSO for preparing dye stock solutions, and a detailed labeling protocol.

Biotium offers ViaFluor™ kits in 3 colors: ViaFluor™ 405, ViaFluor™ 488, and ViaFluor™ 568. More colors will follow, so check our website at www.biotium.com for up to date product information.

Ordering Information

Catalog numbers for ViaFluor™ Cell Proliferation Kits			
Kit name	Laser line	Detection channel	Catalog number
ViaFluor™ 405	405 nm	Pacific Blue	30068
ViaFluor™ CFSE	488 nm	FITC	30050
ViaFluor™ 568	488 or 561 nm	PE-TexasRed	30080

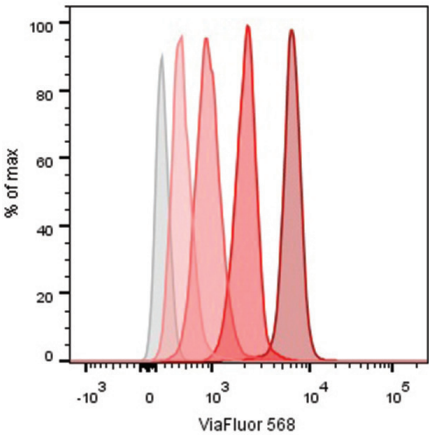


Figure 5. ViaFluor™ Cell Proliferation dyes stably label cells and can be used to track cell divisions over time, in vivo or in culture. Jurkat cells were treated with ViaFluor™ 568 and then maintained in cell culture for various amounts of time. From right to left: 0 days (dark red), 1 day (red), 2 days (light red), 3 days (pink). Unstained cells are shown in gray. As cells divide, each daughter cell receives approximately half of the dye.

Dyes for cell cycle analysis

RedDot™1 is a novel far red nuclear stain developed at Biotium. RedDot™1 is a live cell stain similar to DRAQ5™ that can be used for cell cycle distribution analysis (Figure 6). Unlike with Propidium Iodide, RedDot™1 cell cycle analysis does not require an RNase step. RedDot™1 can also be used as a far-red nuclear counterstain for live cells in microscopy.

Biotium also carries the classic dye Propidium Iodide (PI) for cell cycle analysis.

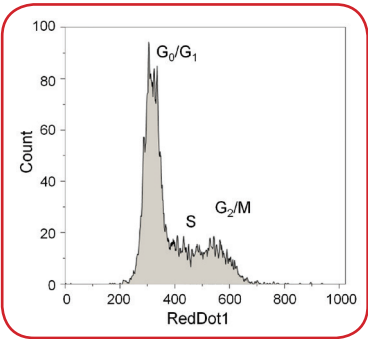


Figure 6. RedDot1 staining for cell cycle distribution analysis. Live Jurkat cells were stained with 1X RedDot1 for 30 minutes at 37°C, then analyzed using a BD LSRII flow cytometer with 633 nm excitation and 710/50 BP emission filter. Image courtesy of Philip Hexley, Shriners Flow Cytometry Core Facility, Shriners Hospital for Children and University of Cincinnati.

Ordering Information

Catalog No.	Product Description	Unit Size
40060-1	RedDot™1	1 mL
40060	RedDot™1	250 uL
40060-T	RedDot™1	25 uL
40016	Propidium Iodide (PI)	100 mg
40017	Propidium Iodide (PI), 1 mg/mL in water	10 mL

RedDot™1 is covered by pending U.S. and international patents. DRAQ5™ is a trademark of Biostatus Limited.

MitoView™ Dyes

Loss of mitochondrial membrane potential is a hallmark for apoptosis. It is an early event preceding phosphatidylserine externalization and coinciding with caspase activation. Biotium offers our MitoView™ Blue and MitoView™ 633 dyes for membrane potential-sensitive staining of mitochondria in microscopy or flow cytometry (Figure 7). We also offer MitoView™ Green, a membrane-potential independent mitochondrial dye that can be used to image mitochondria following mitochondrial depolarization, or after fixation.

JC-1 Mitochondrial Membrane Potential Detection Kit

In healthy cells, JC-1 dye aggregates in mitochondria as a function of membrane potential, resulting in red fluorescence with brightness proportional to the membrane potential. Conversely, in apoptotic and necrotic cells with diminished mitochondrial membrane potential, JC-1 exists in a green fluorescent monomeric form in the cytosol, allowing of cell viability to be assessed by measuring the ratio of red to green fluorescence by flow cytometry or fluorescence plate reader.

We also offer a selection of classic potentiometric mitochondrial stains in a variety of fluorescent colors.

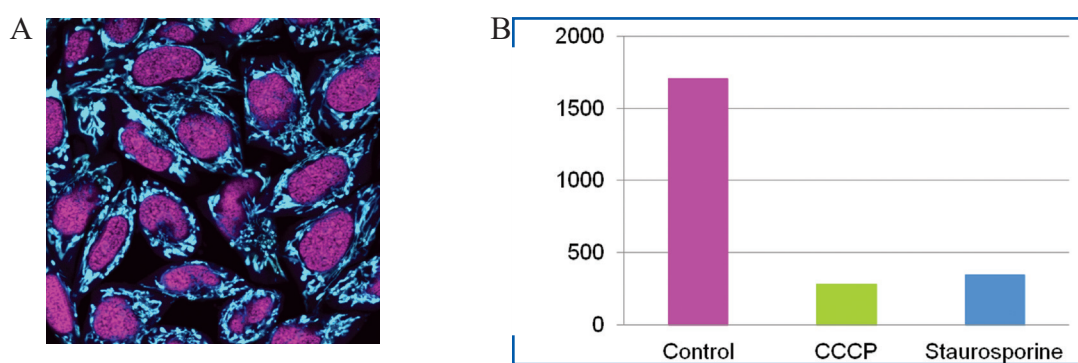


Figure 7. A) HeLa cells stained with MitoView™ Blue (cyan) and RedDot™ 1 far-red nuclear stain (magenta). B) Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

Ordering Information

MitoView™ Dyes	Color	Ex/Em	Mitochondrial Membrane Potential Dependent?	Catalog No.	Size
MitoView™ Blue	Blue	398/440 nm	Yes	70052	20 x 50 ug
MitoView™ 633	Far-red	622/648 nm	Yes	70055	20 x 50 ug
MitoView™ Green	Green	490/523 nm	No	70054	20 x 50 ug
Classic mitochondrial stains	Color	Ex/Em	Mitochondrial Membrane Potential Dependent?	Catalog No.	Size
JC-1 (chloride salt)	Green/Red	510/527 nm (cytoplasm) 585/590 nm (polarized mitochondria)	Yes	70011	5 mg
JC-1 (iodide salt)	Green/Red	510/527 nm (cytoplasm) 585/590 nm (polarized mitochondria)	Yes	70014	5 mg
Rhodamine 123	Green	505/534 nm	Yes	70010	50 mg
TMRM	Red	548/573 nm	Yes	70017	25 mg
TMRE	Red	549/574 nm	Yes	70016	25 mg
TMRE, 2 mM in DMSO	Red	549/574 nm	Yes	70005	0.5 mL

Assay Kits	Color	Ex/Em	Assay	Catalog No.	Size
NucView™ 488 and MitoView™ 633 Apoptosis Kit	Green/Red	500/530 nm (caspase-3) 622/648 nm (polarized mitochondria)	Two color detection caspase-3 activity and mitochondrial potential; see page 5 for more details	30062	100 assays
JC-1 Mitochondrial Membrane Potential Detection Kit	Green/Red	510/527 nm (cytoplasm) 585/590 nm (polarized mitochondria)	Two-color detection mitochondria polarization/depolarization	30001	100 assays
MCB Glutathione Detection Kit	Blue	394/490 nm	Detection of cellular glutathione	30019	100 assays

NucView™ Caspase-3 Substrates for real-time detection of caspase-3 activity in intact cells

Proteolysis of cellular substrates by caspase-3 results in the morphological and biochemical features of apoptosis. NucView™ 488 Caspase-3 Substrate is a novel cell membrane-permeable fluorogenic caspase substrate designed for detecting caspase-3 activity in real time.

Traditional fluorogenic caspase substrates require cell lysis and cannot be used to measure caspase activity in live cells. Fluorescently-labeled caspase inhibitor assay (FLICA) reagents can enter live cells to detect caspase activity, but because the fluorescent probes are also irreversible caspase inhibitors, they cannot be used to follow caspase activity in real time.

NucView™ Caspase-3 Substrates consist of a fluorogenic DNA dye and a DEVD substrate moiety specific for caspase-3. The substrate, which is initially not fluorescent and nonfunctional as a DNA dye, crosses the cell membrane to enter the cytoplasm, where it is cleaved by caspase-3 to form a high-affinity DNA dye. The released dye can bind DNA, resulting in bright nuclear fluorescence (Figure 8), allowing caspase-3 activity to be monitored in individual intact cells in real time. NucView™ substrates also can be used in a rapid and convenient homogenous end-point assay.

We offer green fluorogenic NucView™ 488 Caspase-3 Substrate and kits, validated in more than a hundred published studies and cell types. We also offer blue fluorogenic NucView™ 405 Caspase-3 Substrate for confocal microscopy or flow cytometry using the 405 nm laser line, and orange fluorogenic NucView™ 530 Caspase-3 Substrate for multi-color flexibility.

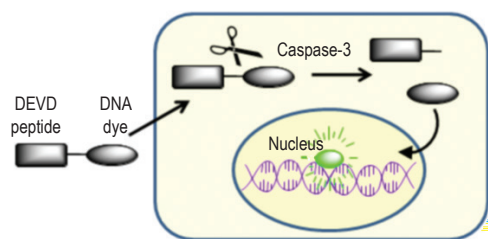


Figure 8. Principal of apoptosis detection using NucView™ Caspase-3 Substrates.

NucView™ Caspase-3 Substrates

- **Bifunctional:** allow caspase-3 detection and visualization of apoptotic nuclear morphology
- **Do not interfere with caspase-3 activity, allowing real time caspase-3 monitoring**
- **Fast staining in cell culture medium with no wash required for imaging or flow cytometry**
- **Tolerant of formaldehyde fixation and permeabilization**
- **Detectable by fluorescence microscopy, flow cytometry, or fluorescence microplate reader**
- **For use in adherent or suspension cells**

NucView™ 405 timecourse

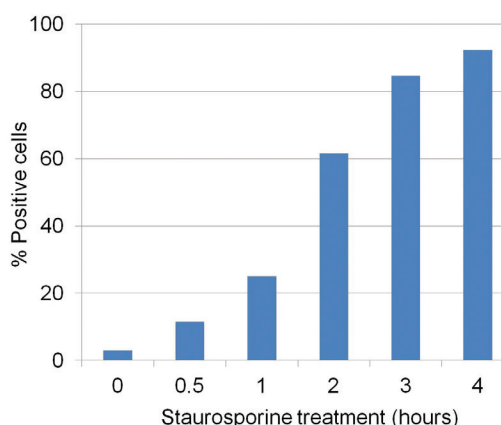


Figure 9. Flow cytometry analysis of Jurkat cells treated with staurosporine to induce apoptosis. Cells were treated with staurosporine for the indicated time, then stained with NucView™405. Fluorescence was analyzed on a BD LSRII flow cytometer. As apoptosis progresses over time in staurosporine, the number of positive cells increases.

Now, more color options

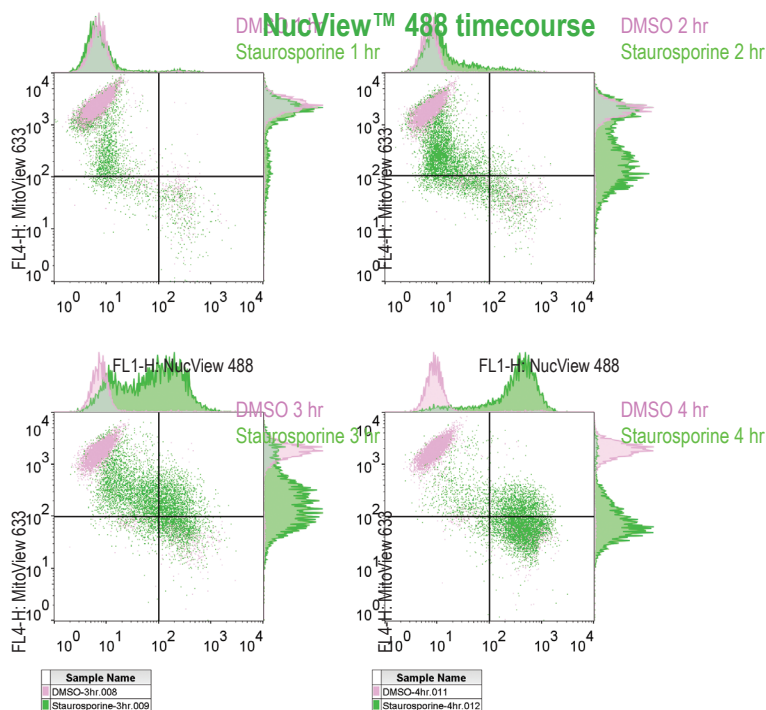
NucView™ 488: Green fluorogenic substrate (Ex/Em 504/534 nm), tested in more than 100 cell lines and publications*

NucView™ 405: Blue fluorogenic substrate (Ex/Em 429/469 nm) for flow cytometry or confocal microscopy with the 405 nm laser line

NucView™ 530: Orange fluorogenic substrate (Ex/Em 528/563 nm) for microscopy or flow cytometry in the Cy®3/R-PE channel

Ordering Information

Caspase Substrates and Detection Kits	Catalog no.	Unit size
NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10402	100 uL
NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in PBS	10403	100 uL
NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10405-T	10 uL trial size
NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10405	100 uL
NucView™ 530 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10406-T	10 uL trial size
NucView™ 530 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10406	100 uL
NucView™ 488 Caspase-3 Assay Kit for Live Cells	30029-T	25 assay trial size
NucView™ 488 Caspase-3 Assay Kit for Live Cells	30029	100 assays
Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™594 Annexin V	30067	50 assays
Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™640R Annexin V	30073	50 assays
NucView™ 488 and MitoView™ 633 Apoptosis Kit	30062	100 assays
NucView™ 488 and RedDot™2 Apoptosis & Necrosis Kit	30072	100 assays
Caspase-3 DEVD-R110 Fluorometric & Colorimetric Assay Kit	30008-1	25 assays
	30008-2	100 assays
Caspase-3 DEVD-R110 Fluorometric HTS Assay	30009-1	10 assays
	30009-2	100 assays
	30009-3	1000 assays
Ac-DEVD-AMC	10202	5 mg
Ac-DEVD-CHO Caspase-3 Inhibitor	10404-1	1 mg
	10404	5 mg



FL1-H: NucView 488

FL1-H: NucView 488

Figure 10. 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 (FL1, x-axis) increases and mitochondrial membrane potential measured by MitoView™ 633 staining (FL4, y-axis) decreases.

Annexin V conjugates

Annexin V is a 35-36 kDa protein that has a 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 (Figure 11) 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 near-infrared CF dye conjugates of Annexin V are supplied lyophilized and preservative-free, and are suitable for in vivo imaging.

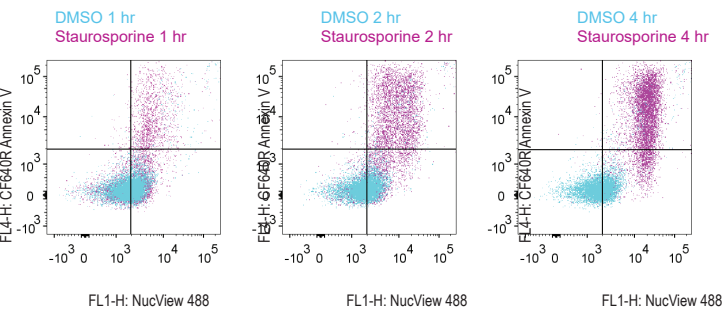


Figure 11. Jurkat cells were treated with staurosporine to induce apoptosis (pink), or with DMSO as a negative control (blue) for the times indicated, then stained for 15 minutes at room temperature with NucView™ 520 Caspase-3 Substrate (FL1-H, x-axis) and CF™640R Annexin V (FL4-H, y-axis) in cell culture medium prior to analysis using a BD LSRII flow cytometer. See pp. 4-5 for more information in NucView™ Substrates.

Ordering Information

Annexin V Conjugates	Ex/Em (nm)	Catalog number	Unit size
Annexin V, CF™350, 50 ug/mL	347/448	29012	0.5 mL
Annexin V, CF™405M, 50 ug/mL	408/452	29009	0.5 mL
Annexin V, CF™488A, 50 ug/mL	490/515	29005	0.5 mL
Annexin V, CF™555, 50 ug/mL	555/565	29004	0.5 mL
Annexin V, CF™568, 50 ug/mL	562/583	29010	0.5 mL
Annexin V, CF™594, 50 ug/mL	593/614	29011	0.5 mL
Annexin V, CF™633, 50 ug/mL	630/650	29008	0.5 mL
Annexin V, CF™640R, 50 ug/mL	642/662	29014	0.5 mL
Annexin V, CF™647, 50 ug/mL	650/665	29003	0.5 mL
Annexin V, CF™680, lyophilized	681/698	29007	25 ug
Annexin V, CF™750, lyophilized	755/777	29006	25 ug
Annexin V, CF™770, lyophilized	770/797	29046	25 ug
Annexin V, CF™790, lyophilized	784/806	29047	25 ug
Annexin V, FITC, 50 ug/mL	490/525	29001	0.5 mL
Annexin V, R-PE	496, 546, 565/578	29045-100 uL	20 assays
Annexin V, R-PE	496, 546, 565/578	29045-500 uL	100 assays
Annexin V, APC	633, 640/660	29057-100 uL	20 assays
Annexin V, APC	633, 640/660	29057-500 uL	100 assays
Annexin V, Texas Red®, 50 ug/mL	596/615	29002	0.5 mL
Annexin V, biotin, 50 ug/mL	N/A	29013	0.5 mL
5X Annexin V Binding Buffer	N/A	99902	15 mL

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

A kit composed of CF™488 Annexin V paired with red fluorescent propidium iodide or far-red fluorescent 7-AAD for detection of necrotic and late apoptotic cells with compromised membrane integrity by fluorescence microscopy or 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 blue fluorescent Hoechst 33342 DNA dye for visualizing the healthy cells.

Dual apoptosis assay kits

Annexin V conjugated to our deep red CF™594 or far-red CF™640R dyes 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 pages 8-9 for more information on NucView™ substrates).

Ordering Information

Apoptosis and Necrosis Detection Kits	Catalog number	Unit size
Dual Apoptosis Assay with NucView™ 488 and CF™594 Annexin V	30067	50 assays
Dual Apoptosis Assay with NucView™ 488 and CF™640R Annexin V	30073	50 assays
Apoptosis & Necrosis Quantitation Kit Plus	30065	50 assays
Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus	30066	50 assays
CF™488A Annexin V and 7-AAD Apoptosis Kit	30060	100 assays
CF™488A Annexin V and PI Apoptosis Kit	30061	100 assays

AccuEasy™ Kit for cell surface staining of adherent cells for flow cytometry

AccuEasy™ is a novel method for staining adherent cells for flow cytometry analysis. Conventional flow cytometry staining protocols for cell surface markers on adherent cells require detachment of cells from their culture surface before performing antibody staining. Unfortunately, cell detachment introduces a significant stress to cells which can alter the expression of cell surface markers.

The AccuEasy™ Flow Cytometry Kit provides a simpler, more accurate and more sensitive method for detecting cell surface markers on adherent cells. AccuEasy™ allows you to detect cell surface marker expression on cells in their native adherent state, avoiding potential loss of cell surface markers during cell detachment. The AccuEasy™ method also eliminates laborious centrifugation steps, increasing throughput. As shown in Figure 12, the AccuEasy™ Kit increases the detection sensitivity for a variety of cell surface markers in comparison to conventional staining after cell detachment.

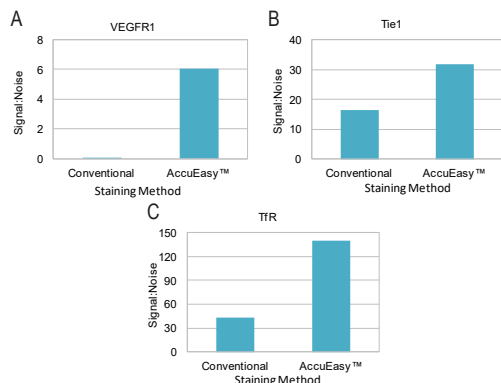


Figure 12. Flow cytometry analysis of cell surface markers on adherent cells for cells stained after detachment from the culture plate (conventional method) or cells stained using the AccuEasy™ method. The AccuEasy method generated higher signal to noise ratios (geometric mean fluorescence for marker antibody over isotype control antibody) for all three surface markers tested. A. EA.hy926 human endothelial cells stained with mouse monoclonal antibody against VEGF Receptor 1 (VEGFR1) followed by PE conjugated anti-mouse IgG. B. EA.hy926 cells stained with mouse monoclonal antibody against Tie1 followed by PE conjugated anti-mouse IgG. C. HeLa cells stained with Mix-n-Stain™ CF™488A labeled mouse monoclonal antibody against transferrin receptor (TfR).

Mini Cell Scrapers

For detachment of adherent cells from multi-well plates, Biotium offers Mini-Cell Scrapers for 96-, 48- and 24-well plates. The polyethylene scrapers are 0.5 cm in width and 6 cm in length (Figure 13), and are disposable & sterile.

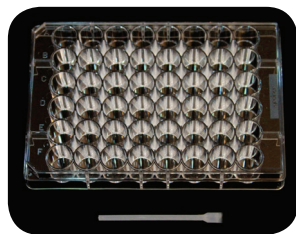


Figure 13. Mini Cell Scraper shown next to a 48-well plate for scale.

Buffers for Flow Cytometry

The Flow Cytometry Fixation/Permeabilization Kit contains optimally formulated buffers for fixation and permeabilization of suspension cells for immuno-staining of intracellular antigens for analysis by flow cytometry. Fixation, permeabilization and permeabilization/blocking buffers also are available separately. For your convenience, Biotium offers a selection of commonly used blocking agents and detergents for immunofluorescence staining.

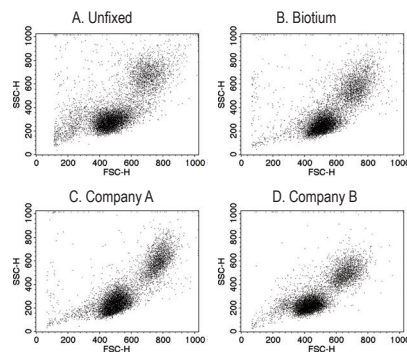


Figure 14. Comparison of Biotium's Flow Cytometry Fixation/Permeabilization Kit with leading competitors' fixation/permeabilization kits. Primary human PBMC were left unfixed (A) or fixed and permeabilized according to kit manufacturer's protocols (B-D) and analyzed on a BD FACSCalibur flow cytometer for forward/side scatter profiles.

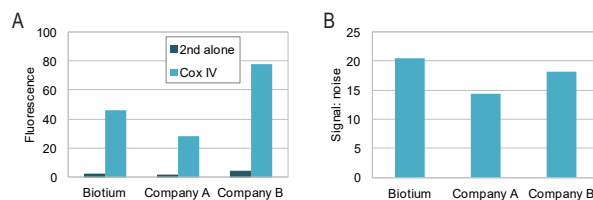


Figure 15. Comparison of immunofluorescence staining for an intracellular antigen using Biotium's Flow Cytometry Fixation/Permeabilization Kit compared to leading competitors' kits. Jurkat cells were fixed and permeabilized according to kit protocols and stained with rabbit anti-COXIV antibody followed by CF™488A-conjugated goat anti-rabbit secondary antibody and analyzed on a BD FACSCalibur flow cytometer in channel FL1. A. Fluorescence signal with and without primary antibody. Bars represent the geometric mean fluorescence of the cell populations. B. Signal to noise ratio.

Ordering Information

Catalog No.	Product Description	Unit Size
30069	AccuEasy™ Flow Cytometry Kit	Kit
22003	Mini Cell Scrapers	Pack of 200
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
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

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Biotium, Inc.

Toll Free: 800-304-5357

Phone: 510-265-1027

Fax: 510-265-1352

General Inquiries
order@biotium.com

Quotes and Ordering
order@biotium.com

Technical Support
techsupport@biotium.com