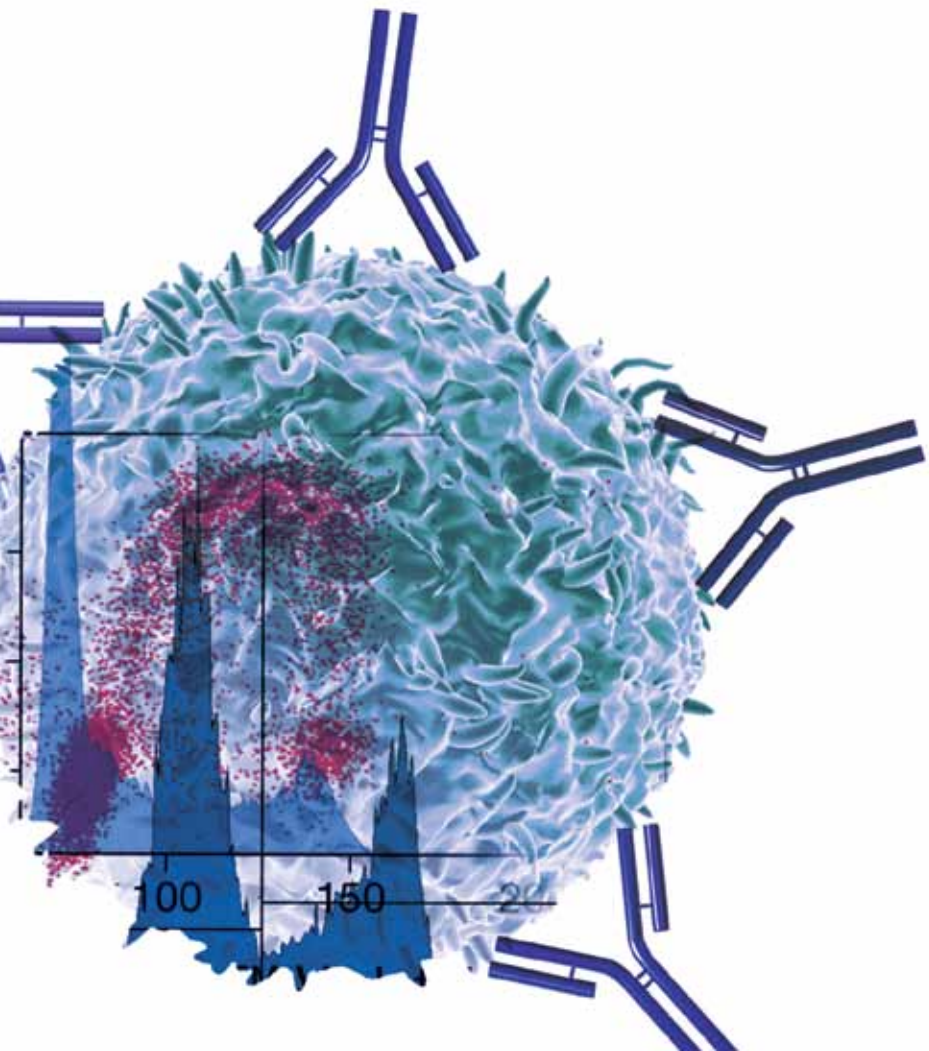
The flow cytometry violet laser resource guide

Molecular Probes® violet laser–excitable reagents



invitrogen™
by life technologies™

Put your flow cytometer's violet laser to work

Our violet-excitable reagents give you:

- → Multiplexing capability to maximize the potential of your flow cytometer
- → Unique cell biology assays for vitality, viability, cell cycle analysis, cell proliferation, and apoptosis
- → The option to collect routine data off the violet laser, freeing other channels
- → Many choices in primary and secondary detection using violet excitation (Pacific Blue™ and Pacific Orange™ dyes and Qdot® nanocrystal conjugates)

Primary antibodies and secondary detection

Pacific Blue™ and Pacific Orange™ dyes

With an emission maximum at 551 nm, Pacific Orange™ dye is fully compatible with Pacific Blue™ dye (emission maximum 455 nm), giving you the option of multiparametric analysis with the violet laser (Figure 1). The Pacific Orange™ and Pacific Blue™ product lines include Zenon® antibody labeling kits for mouse IgG as well as direct conjugates with mouse and rabbit IgG and streptavidin.

Qdot® nanocrystals

The extraordinary optical properties of Qdot® nanocrystals make them ideal labels for flow cytometry. Their extremely bright fluorescence emission makes them well suited for the detection of low-abundance extracellular proteins (Figure 2). Efficient optical excitation is possible using the 405 nm violet excitation light source. In addition, the narrow, symmetric emission profiles of the Qdot® nanocrystal conjugates require substantially lower compensation, enabling better, more efficient multicolor assays using the violet laser (Figure 3). A wide range of markers directly conjugated with Qdot® nanocrystals are available, in addition to a host of streptavidin conjugates to detect biotinylated antibodies.

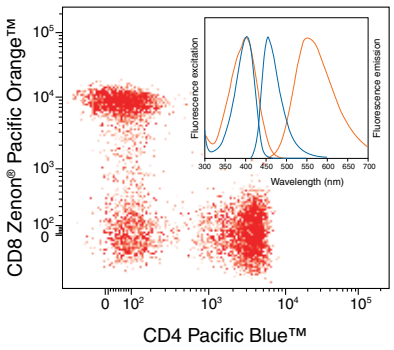


Figure 1. Violet excitation for multiplexing. Human mononuclear cells were labeled with Pacific Blue™ anti-CD4 and Zenon® Pacific Orange™ anti-CD8 antibodies. Analysis was performed on the BD LSR™ II flow cytometer using 405 nm excitation and 450/50 nm and 585/42 nm emission filters. Inset shows spectra for Pacific Blue™ dye (blue lines) and Pacific Orange™ dye (orange lines).

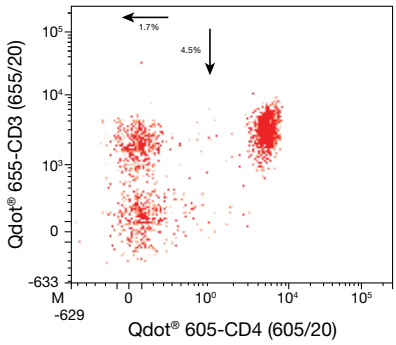


Figure 2. Multicolor analysis of CD3-positive and CD4-positive cell populations using Qdot® primary antibody conjugates. Human PBLs were stained with Qdot® 605 anti-CD4 and Qdot® 655 anti-CD3 antibodies. Lymphocytes were analyzed for fluorescence using violet diode laser excitation and 605/20 nm and 655/20 nm emission filters. Samples were run on a BD LSR™ II flow cytometer. Plots are gated on lymphocytes by side scatter/CD45. Axes are labeled with the band-pass filters used; plots are labeled with compensation values (arrows).

Ordering information

Primary antibodies for human markers

Target	Clone	Isotype	Pacific Blue™	Pacific Orange™	Qdot® 605	Qdot® 655	Qdot® 705	Qdot® 800
CD2	S5.5	Mouse IgG2a			Q10172			
CD3	UCHT1	Mouse IgG2a	CD0328	CD0330	Q10054			
CD3	S4.1	Mouse IgG2a	MHCD0328			Q10012		
CD4	S3.5	Mouse IgG2a	MHCD0428	MHCD0430	Q10008	Q10007	Q10060	
CD8	3B5	Mouse IgG2a	MHCD0828	MHCD0830	Q10009	Q10055	Q10059	
CD10	MEM-78	Mouse IgG1			Q10153			Q10154
CD11c	BU-15	Mouse IgG1	MHCD11c28					
CD14	TüK4	Mouse IgG2a	MHCD1428	MHCD1430	Q10013	Q10056		Q10064
CD15	H130	Mouse IgM	MHCD1528	MHCD1530				
CD16	3G8	Mouse IgG1	MHCD1628	MHCD1630				
CD19	SJ25-C1	Mouse IgG1	MHCD1928		Q10306	Q10179		
CD20	HI47	Mouse IgG3		MHCD2030		Q10305		
CD27	CLB-27/1	Mouse IgG2a			Q10065	Q10066		
CD38	HIT2	Mouse IgG1			Q10053	Q10057		
CD45	HI30	Mouse IgG1	MHCD4528	MHCD4530	Q10051	Q10155	Q10062	Q10156
CD45RA	MEM-56	Mouse IgG2b	MHCD45RA28		Q10047	Q10069		
CD56	MEM-188	Mouse IgG2a			Q10307			
CD62L	Dreg 56	Mouse IgG1	MHCD62L28					
CD64	10.1	Mouse IgG1	CD6428					
CD95	DX2	Mouse IgG1	MHCD9528					
HLA-DR	Tü36	Mouse IgG2a	MHLDR28	MHLDR30	Q10052			Q10063
IFN-γ	B27	Mouse IgG1	MHCIFG28					
TNFα	MP9-20A4	Rat IgG1	RHTNFA28					
ZAP-70	1E7.2	Mouse IgG1	MHZAP7028					

Primary antibodies for mouse markers

CD3	500A2	Hamster IgG	HM3428					
CD3	124-2C11	Hamster IgG			Q10090			
CD4	RM4-5	Rat IgG2a	MCD0428	MCD0430	Q10092			
CD8a	5H10	Rat IgG2b	MCD0828	MCD0830				
CD11b	M1/70.15	Rat IgG2b	RM2828					
CD25	Pc61-53	Rat IgG1	RM6028					
CD31	390	Rat IgG2a	RM5228					
CD45	30-F11	Rat IgG2b	MCD4528	MCD4530				
CD45R (B220)	RA3-6B2	Rat IgG2a	RM2628	RM2630		Q10176		
CD62L	MEL-14	Rat IgG2a	RM4328					
CD69	H1-2F3	Hamster IgG	HM4028					
F4/80	BM8	Rat IgG2a	MF48028	MF48030				
Ly-6A/E (Sca-1)	D7	Rat IgG2a	MSCA28					
Ly-6C/G (Gr-1)	RB6-8C5	Rat IgG2b	RM3028	RM3030				
NK1.1	PK-136	Mouse IgG2	MM6628					
TCRαβ	H57-597	Hamster IgG	HM3628					
TNFα	MP6-XT22	Rat IgG2a	RM90128					
ZAP-70	1E7.2	Mouse IgG1	MHZAP7028					

Isotype controls

Hamster IgG	NA	Hamster IgG	HM28					
Mouse IgG1	NA	Mouse IgG1	MG128	MG130	Q10073			Q10298
Mouse IgG2a	NA	Mouse IgG2a	MG2a28	MG2a30	Q10014	Q10015	Q10076	Q10075
Mouse IgG2b	NA	Mouse IgG2b	MG2b28		Q10074			
Rat IgG2a	NA	Rat IgG2a		R2a30				
Rat IgG2b	NA	Rat IgG2b	R2b28	R2b30				

All antibodies listed in this table are for research use only.

continued

Ordering information, continued

Streptavidin and secondary antibody conjugates

Product	Host	Form	Pacific Blue™	Pacific Orange™	Qdot® 565	Qdot® 605	Qdot® 625	Qdot® 655	Qdot® 705	Qdot® 800
Anti-Mouse IgG	Goat	Whole Ab	P10993							
Anti-Mouse IgG*	Goat	Whole Ab	P31582							
Anti-Mouse IgG	Goat	$F(ab')_2$	P31581	P31585	Q11031MP	Q11001MP	A10195*	Q11021MP	Q11061MP	Q11071MP
Anti-Rabbit IgG	Goat	Whole Ab	P10994	P31584						
Anti-Rabbit IgG	Goat	$F(ab')_2$			Q11431MP	Q11401MP	A10194*	Q11421MP	Q11461MP	Q11471MP
Streptavidin	NA	NA	S11222	S32365	Q10131MP	Q10101MP	A10196	Q10121MP	Q10161MP	Q10171MP

* Highly cross-adsorbed.

Antibody and Zenon® labeling kits

Product	Pacific Blue™	Pacific Orange™	Qdot® 565	Qdot® 605	Qdot® 625	Qdot® 655	Qdot® 705	Qdot® 800
APEX™ Antibody Labeling Kit	A10478							
Monoclonal Antibody Labeling Kit	P30013	P30014	Q22031MP	Q22001MP	A10197	Q22021MP	Q22061MP	Q22071MP
Protein Labeling Kit	P30012	P30016						
Zenon® Mouse IgG1 Labeling Kit	Z25041	Z25256						
Zenon® Mouse IgG2a Labeling Kit	Z25156	Z25257						

Reactive dyes

Product	Reactive moeity	Reactivity	Ex/Em	Quantity	Cat. No.
Pacific Blue™	Succinimidyl ester	Amines	410/455 nm	5 mg	P10163
Pacific Blue™	Maleimide	Thiols (mercaptans, sulfhydryls)	410/455 nm	1 mg	P30506
Pacific Orange™	Succinimidyl ester	Amines	404/553 nm	1 mg	P30253
Pacific Orange™	Maleimide	Thiols (mercaptans, sulfhydryls)	404/553 nm	1 mg	P30507

Compensation beads

Product	Quantity	Cat. No.
AbC™ Anti-Mouse Bead Kit, for mouse antibody capture	100 tests	A10344
AbC™ Anti-Rat/Hamster Bead Kit, for rat/hamster antibody capture, for flow cytometry	100 tests	A10389

Cell counting

Product	Laser	Emission maxima	Quantity	Cat. No.
CountBright™ Absolute Counting Beads	UV–635 nm	385–800 nm	5 mL	C36950

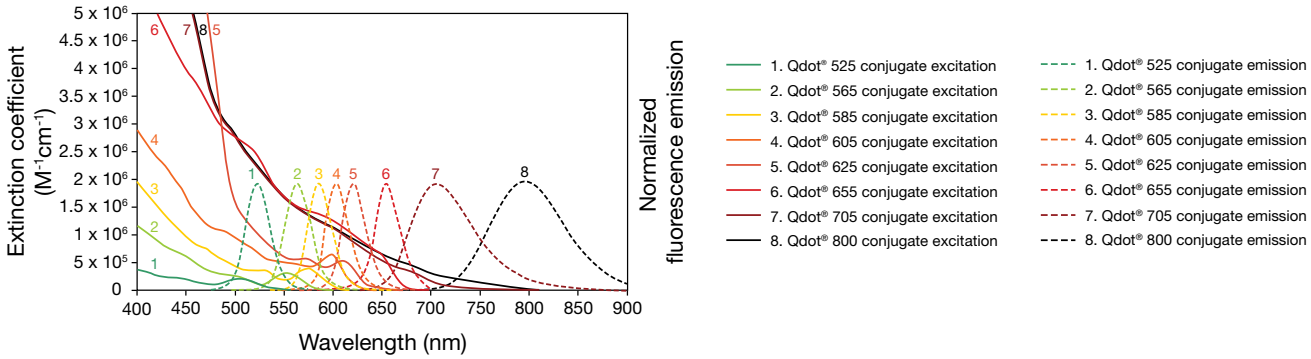


Figure 3. Absorption and emission profiles of Qdot® nanocrystals. Qdot® nanocrystals are characterized by broad absorption spectra and narrow, symmetrical, and discrete emission profiles.

Cell cycle analysis

Cell cycle analysis in live cells using Vybrant® DyeCycle™ Violet Stain

Vybrant® DyeCycle™ Violet is used for DNA content analysis in live cells. Vybrant® DyeCycle™ Violet Stain is DNA selective, cell membrane–permeant, and nonfluorescent until bound to double-stranded DNA. Cell cycle analysis based on Vybrant® DyeCycle™ Violet staining results in a clear pattern of distribution: G_0/G_1 phase (one set of paired chromosomes per cell), S phase (DNA synthesis with variable amount of DNA), and G_2/M phase (two sets of paired chromosomes per cell, prior to cell division) (Figure 4). Vybrant® DyeCycle™ Violet Stain is well suited for the 405 nm laser line but can also be used with UV excitation, having an emission peak at ~440 nm. This live-cell dye allows the simultaneous co-staining of live cells for other parameters and offers the possibility of cell sorting based on DNA content. Vybrant® DyeCycle™ Violet Stain has also been shown to identify stem cell side populations and early progenitors in mammalian hematopoietic tissues using the violet laser (Figure 5).

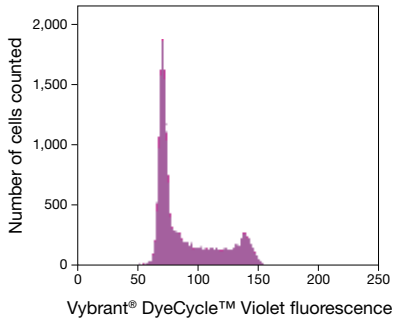


Figure 4. Cell cycle analysis using Vybrant® DyeCycle™ Violet Stain. Histogram of live Jurkat cells stained with Vybrant® DyeCycle™ Violet Stain showing DNA content distribution. G_0/G_1- and G_2/M-phase histogram peaks are separated by the S-phase distribution. Violet 405 nm excitation was used with a 440/40 nm bandpass filter.

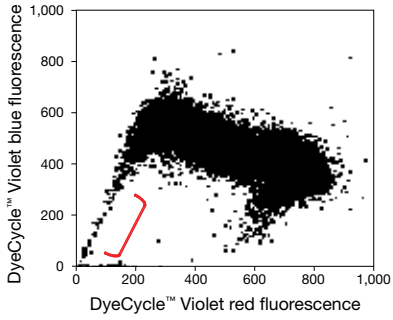


Figure 5. DyeCycle™ Violet Stain stem cell side population analysis in human cord blood. Cells were incubated with 10 µM DyeCycle™ Violet Stain for 90 min at 37°C, then washed and stored on ice until analysis. Results showed a recognizable side population of cells (red bracket) following violet laser excitation. Data courtesy of Dr. William Telford, NIH.

Cell cycle analysis in fixed cells using FxCycle™ Violet Stain

FxCycle™ Violet Stain is a violet laser–excited dye used for cell cycle analysis in fixed cells. Using FxCycle™ Violet for cell cycle analysis increases the ability to multiplex by freeing up the 488 nm and 633 nm lasers for other cellular analyses such as immunophenotyping, apoptosis analysis, and/or dead cell discrimination (Figure 6). Because of its narrow emission spectra, FxCycle™ Violet Stain overlaps less with other fluorescent channels than do other commonly used dyes (propidium iodide and 7-AAD), resulting in minimal compensation requirements and more accurate data.

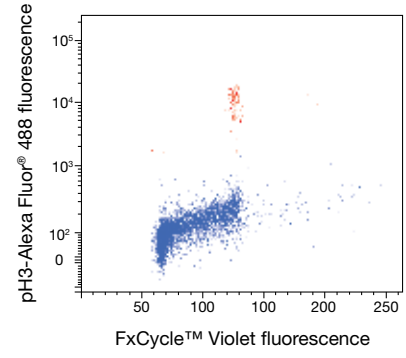


Figure 6. Multiparametric cell cycle and immunophenotypic analysis. TF-1 erythroblast cells were alcohol-fixed overnight, washed, and then suspended in 0.1% Triton® X-100/PBS/1% BSA before staining with anti–histone H3[pS10] purified antibody (Cat. No. 441190G) complexed with Zenon® Alexa Fluor® 488 Rabbit IgG labeling reagent and FxCycle™ Violet Stain. The pH3 signal (red) identifies cells that are in mitosis.

Ordering information

Product	Cell status	Ex/Em	Assays	Cat. No.
Vybrant® DyeCycle™ Violet Stain	Live	369/437	200	V35003
FxCycle™ Violet Stain	Fixed	358/461	500	F10347

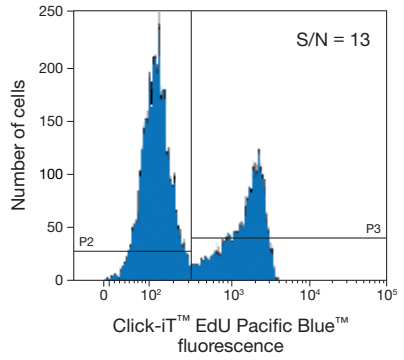


Figure 7. Analysis of cell proliferation using the Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay Kit. Jurkat cells were treated with 10 µM EdU for 2 hr and detected according to the staining protocol. Cells were labeled with Pacific Blue™ azide using 405 nm excitation with a 450/50 nm bandpass; the signal-to-background ratio (S/N) was calculated by dividing the median fluorescence intensity of the Click-iT™ EdU–positive population (P3) by the median fluorescence intensity of the nonproliferating population (P2) as indicated by the markers.

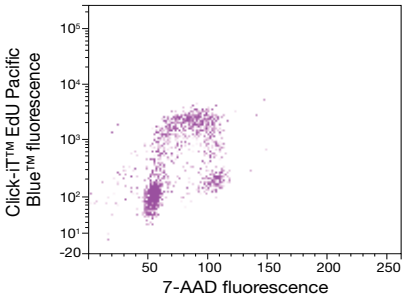


Figure 8. Flow cytometric analysis of cell proliferation using Click-iT™ technology. Jurkat cells were treated with 10 µM EdU for 2 hr, then fixed and permeabilized, stained with the click reaction, washed, and counterstained for cell cycle analysis using 7-aminoactinomycin D (7-AAD). Cells were analyzed using a flow cytometer with 405 nm excitation and 450/50 nm bandpass and 675/20 nm bandpass filters.

Analysis of cellular proliferation (DNA synthesis)

Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay Kit

Nucleoside incorporation provides direct measurement of new DNA synthesis. Traditionally, this has been performed by incorporating the nucleoside analog bromodeoxyuridine (BrdU) into DNA, followed by detection with an anti-BrdU antibody. This method requires DNA denaturation (using HCl, heat, or DNase) to expose the BrdU to the antibody—a step that can be lengthy and difficult to perform consistently, with adverse effects on sample quality. The Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay Kit eliminates the need to denature DNA, providing a superior alternative to the standard BrdU antibody–based method for measuring cell proliferation by flow cytometry. The Click-iT™ advantage is in the chemistry—small, unique, and low-background labeling and detection moieties that react specifically and covalently with one another. 5-ethynyl-2′-deoxyuridine (EdU) is a nucleoside analog containing an alkyne. In a copper-catalyzed reaction, the alkyne reacts with a Pacific Blue™ dye–labeled azide, forming a stable covalent bond. The small size of the azide reagents allows efficient access to the DNA without the need for harsh cell treatment, thus simplifying the assay considerably, yet generating the same results (Figures 7 and 8). Click-iT™ EdU labeling is compatible with most fixation protocols.

Ordering information

Product	Ex/Em	Assays	Cat. No.
Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay Kit	410/455	50	A10034

Analysis of cellular proliferation (dye dilution)

CellTrace™ Violet Cell Proliferation Kit

Because of its homogeneous cell labeling and excellent intergenerational resolution, CFSE has been widely used to analyze cell proliferation *in vivo*. Analysis of cell proliferation by CFSE is achieved by dye dilution, whereby each daughter cell has half of the fluorescence intensity of the parent cell following cell division. CFSE produces more homogeneous cellular labeling and, consequently, much better intergenerational resolution than other cell-tracking dyes, such as the membrane marker PKH26. However, because CFSE is based on the fluorescein molecule, cell proliferation experiments on GFP-expressing cells or immunophenotyping with FITC-conjugated antibodies have been impossible to perform in combination with CFSE. Like CFSE, the new Molecular Probes® CellTrace™ Violet Cell Proliferation Kit allows analysis of cell proliferation by dye dilution. However, the violet excitation and blue emission of CellTrace™ Violet will allow the researcher to perform cell proliferation experiments with a wide range of blue-excitable fluorophores, including GFP-expressing cells. CellTrace™ Violet also has better cellular retention than CFSE, enabling resolution of up to 10 generations of cells (Figure 9) with minimal toxic effects.

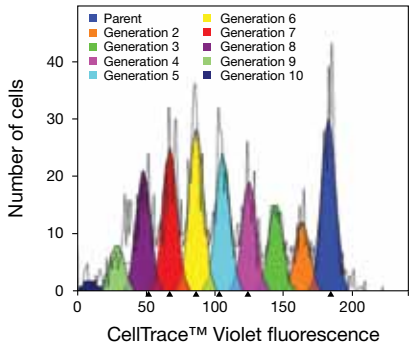


Figure 10. Cell proliferation analysis of Human $CD8^+$ T lymphocytes stained with 10 µM violet laser cell proliferation reagent.

Ordering information

Product	Ex/Em	Assays	Cat. No.
CellTrace™ Violet Cell Proliferation Kit	405/450	180	C34557

Analysis of viability and vitality

For protocols requiring fixation: LIVE/DEAD® Fixable Dead Cell Stains

The fixable dead cell dyes, which covalently interact with available amines, are excluded from the interior of healthy cells. Therefore, the dyes are only able to react with the surface proteins on healthy cells. In contrast, for dead cells, the dyes label proteins throughout the cytoplasm, leaving dead cells with at least 50-fold greater fluorescence than live cells. Because the labeling is covalent, labeled cells can be aldehyde-fixed and permeabilized without losing viability discrimination (Figure 10). Three options are available for use on the violet laser, LIVE/DEAD® Fixable Violet (~460 nm emission), Aqua (~530 nm emission), and Yellow (~575 nm emission) Dead Cell Stains. These reagents are ideal for researchers who want to fix samples before analysis and also maintain dead cell discrimination during intracellular staining (Figure 11).

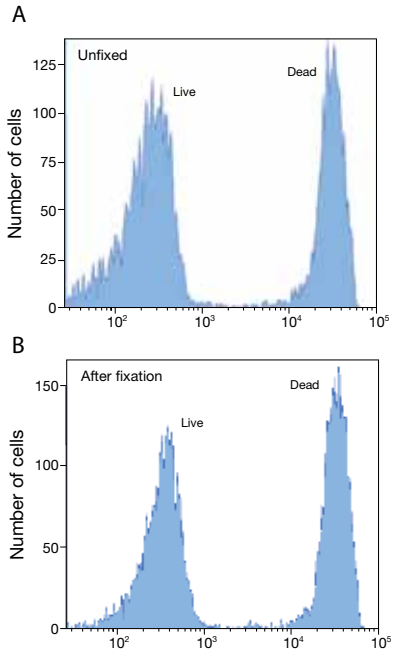


Figure 10. Use of fixable dead cell dyes. The reagents in the LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit were used to differentially stain a mixture of live (left peak) and heat-treated Jurkat cells (right peak). The cells in **(A)** were not fixed; the cells in **(B)** were fixed in 3.7% formaldehyde following the staining reaction. Samples were analyzed by flow cytometry using 405 nm excitation and ~525 nm emission.

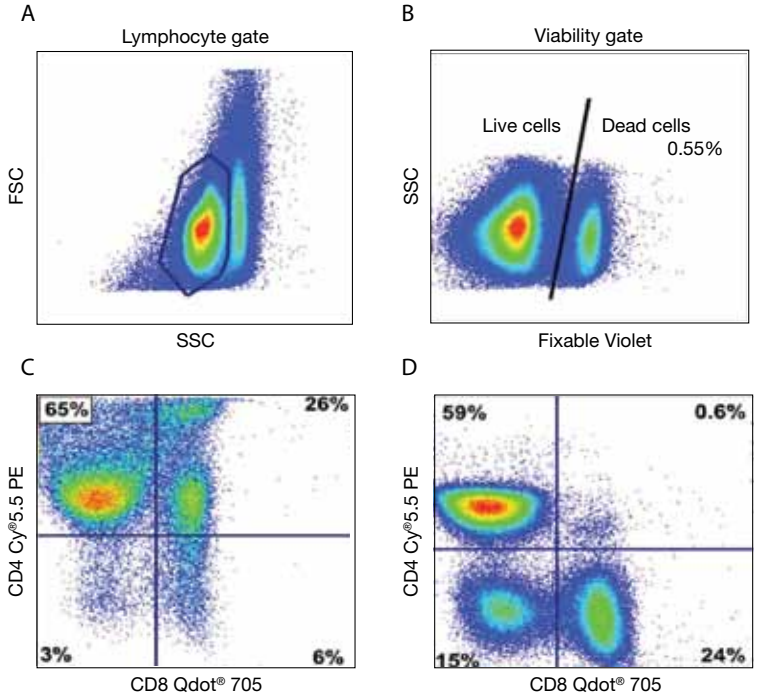


Figure 11. Exclusion of dead cells eliminates staining artifacts from analysis. After the application of a lymphocyte gate **(A)**, live and dead cells were discriminated using LIVE/DEAD® Fixable Violet dye **(B)**. Subsequent analysis of dead **(C)** and live **(D)** cells shows the dramatic difference in apparent phenotypes between the two cell populations.

For protocols without fixation: SYTOX® Blue Dead Cell Stain

Functioning by a mechanism similar to that of propidium iodide (PI), SYTOX® Blue Dead Cell Stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes but will not cross uncompromised cell membranes. After brief incubation with SYTOX® Blue Dead Cell Stain, the nucleic acids of dead cells fluoresce bright blue when excited with 405 nm violet laser light (Figure 12). These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® Blue Dead Cell Stain a simple and quantitative single-step dead cell indicator for use with violet laser–equipped flow cytometers.

For measurements of cell vitality: Calcein Violet AM

Cell vitality as measured by intracellular esterase activity is a recognized parameter of cell health. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein violet AM to the intensely fluorescent calcein violet (excitation/emission 400/452 nm). Calcein violet AM is an optimal dye for this application; utilizing the violet laser allows other laser lines to be used with more conventional markers. Calcein violet AM is available either as a stand-alone reagent or in the LIVE/DEAD® Violet Viability/Vitality Kit, which combines the calcein violet fluorescence with the aqua-fluorescent dead cell reactive dye (Figure 13). This unique kit allows the analysis of both live and dead cells using the violet laser.

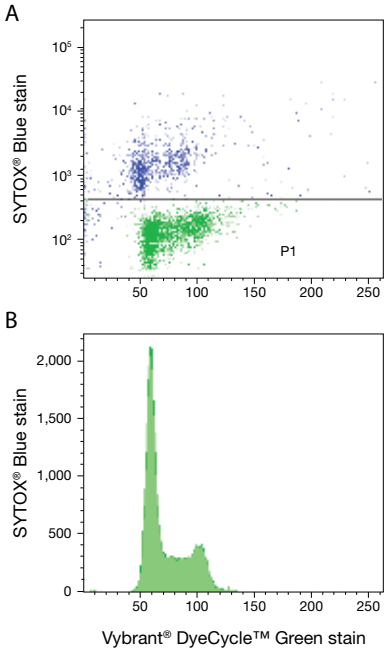


Figure 12. Use of SYTOX® Blue Dead Cell Stain with Vybrant® DyeCycle™ Green Stain. Jurkat cells were stained with Vybrant® DyeCycle™ Green Stain, then stained with the impermeant DNA dye SYTOX® Blue Dead Cell Stain, and analyzed by flow cytometry using 405 nm and 488 nm excitation, gating on SYTOX® Blue–negative cells to eliminate dead cells. Cell cycle was then evaluated using a histogram of Vybrant® DyeCycle™ Green staining.

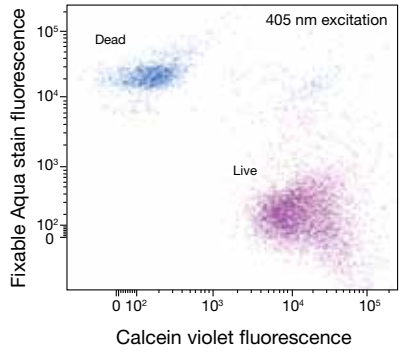


Figure 13. Combination of esterase substrates with dead cell dyes using mixtures of heat-treated and untreated cells. Chinese hamster ovary (CHO) cells were stained according to the protocol in the LIVE/DEAD® Violet Viability/Vitality Kit (calcein violet and fixable violet dead cell stain). Cells were analyzed by flow cytometry using 405 nm excitation.

Ordering information

Product	Cell status	Ex/Em	Assays	Cat. No.
Fixable dead cell stains				
LIVE/DEAD® Fixable Violet Dead Cell Stain Kit	Dead	416/451	200	L34955
LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit	Dead	367/526	200	L34957
LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit	Dead	400/575	200	L34959
LIVE/DEAD® Fixable Dead Cell Sampler Kit	Dead	Various	300	L34960
Compensation beads for fixable stains				
ArC™ Bead Kit, for use with amine reactive dyes, for flow cytometry compensation	NA	NA	100	A10346
Nucleic acid–binding dead cell stains				
SYTOX® Blue Dead Cell Stain, for flow cytometry	Dead	444/480	1,000	S34857
Viability/vitality				
LIVE/DEAD® Violet Viability/Vitality Kit	Live/dead	400/452 (live) 367/526 (dead)	200	L34958
Calcein Violet, AM	Live	400/452	1,000	C34858

Apoptosis assays

Membrane asymmetry

Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry. Fluorescently labeled annexin V conjugates are the most common reagents used to analyze changes in membrane asymmetry. The Pacific Blue™ Annexin V/SYTOX® AADvanced™ Apoptosis Kit provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to the violet-excitable Pacific Blue™ dye, to provide maximum sensitivity, and the red fluorescent SYTOX® AADvanced™ Dead Cell Stain, for identifying necrotic cells based on membrane integrity. Because Pacific Blue™ dye absorbs maximally at 415 nm with fluorescence emission at 455 nm, it is a good choice for violet diode laser excitation in flow cytometry.

Ratiometric membrane asymmetry

The Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit is an assay for use on a violet laser flow cytometer that is an easy, efficient method for the detection of apoptosis. It works well on adherent and suspension cells and correlates with other indicators of apoptosis like caspase detection and changes in mitochondrial membrane potential. It contains a violet ratiometric membrane asymmetry probe, F2N12S, which exhibits an excited-state intramolecular proton transfer (ESIPT) reaction resulting in a dual fluorescence with two emission bands corresponding to 530 nm and 585 nm, producing a two-color ratiometric response to variations in surface charge. Given that apoptosis modifies the surface charge of the outer leaflet of the plasma membrane, the violet membrane asymmetry probe F2N12S can monitor changes in membrane asymmetry that occur during apoptosis through a change in the relative intensity of the two emission bands of the dye (Figure 14). Unlike annexins, this assay does not need any special buffers or wash steps and is less affected by cell membrane damage commonly found during the physical or chemical removal steps when assaying adherent cells, therefore providing better data quality. The ratiometric probe provides self-calibrating, absolute measurement of apoptotic transformation, which is independent of probe concentration, cell size, and instrument variation, such as fluctuations of laser intensity or sensitivity of the detectors.

Membrane permeability

There are some situations in which staining cells with annexin V is not the optimal method for detecting apoptosis. These include assays where cells are sensitive to the high calcium concentrations required for annexin V binding, assays where phosphatidylserine detection on adherent cells is adversely affected by trypsinization, and assays where washing of samples is prohibitive. The violet laser–excited cyanine dye PO-PRO™-1 has been shown to penetrate apoptotic cells because of permeability changes associated with the loss in asymmetry of the plasma membrane. These dyes enter apoptotic cells and bind nucleic acids, while cell-impermeant dead cell stains are excluded. The Violet Membrane Permeability/Dead Cell Apoptosis Kit provides a rapid and convenient assay for apoptosis. The kit contains recombinant PO-PRO™-1 to identify apoptotic cells and the red fluorescent dye 7-AAD for identifying necrotic cells based on membrane permeability (Figure 15).

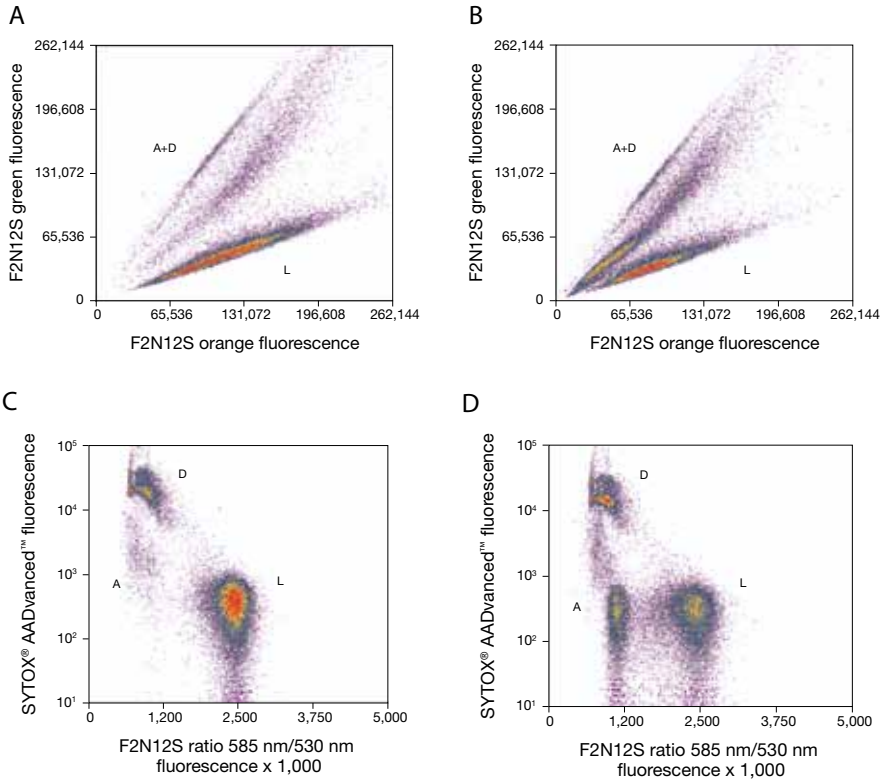


Figure 14. Jurkat cells (T cell leukemia, human) treated with 10 µM camptothecin for 4 hr (panels B and D) or untreated control (panels A and C). Cells were stained according to the protocol. Samples were analyzed on a flow cytometer with 405 nm excitation using 585 nm and 530 nm bandpass filters for F2N12S and 488 nm excitation for SYTOX® AADvanced™ Dead Cell Stain using a 695 nm bandpass filter. In panels **A** and **B**, live cells can be discriminated from apoptotic and dead cells by the relative intensities of the two emission bands from F2N12S. In panels **C** and **D**, SYTOX® AADvanced™ Dead Cell Stain fluorescence is plotted against a derived ratio parameter from the two emission bands (585/530 nm) of F2N12S. A = apoptotic cells, L = live cells, D = dead cells.

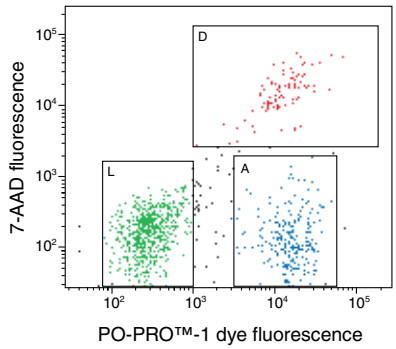
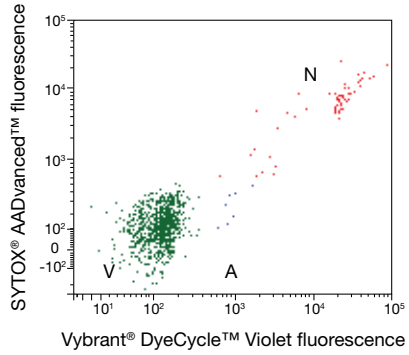


Figure 15. Violet-excited DNA stain for apoptosis detection. Jurkat cells were treated with 10 µM camptothecin for 4 hr and then stained with the reagents in the Violet Membrane Permeability/Dead Cell Apoptosis Kit. Cells were analyzed by flow cytometry using 405 nm and 488 nm excitation. Apoptotic cells **(A)** are clearly distinguished from live cells **(L)** and dead cells **(D)**.

A



B

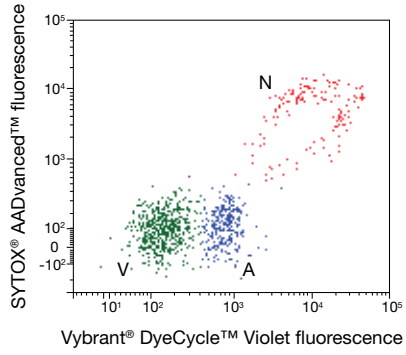


Figure 16. Jurkat cells (human T cell leukemia) treated with 10 µM camptothecin for 6 hr (panel B) or untreated (panel A). Cells were mixed with the reagents in the apoptosis kit with Vybrant® DyeCycle™ Violet/SYTOX® AADvanced™ and analyzed by flow cytometry using 405/488 nm dual excitation. Note that the campthothecin-treated cells (**B**) have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (**A**). A = apoptotic cells, V = viable cells, N = necrotic cells.

Apoptosis assays, continued

Chromatin condensation

Cells undergoing apoptosis display an increase in nuclear chromatin condensation; the compact nuclei become hyperfluorescent when labeled with some nuclear stains. The Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet /SYTOX® AADvanced™ dye provides a rapid and convenient assay for apoptosis based upon fluorescence analysis of the compacted state of the chromatin in apoptotic cells (Figure 16). The kit contains the cell-permeant, violet laser–excited Vybrant® DyeCycle™ Violet Stain and the impermeant, red-fluorescent SYTOX®AADvanced™ Dead Cell Stain. The condensed chromatin of apoptotic cells is stained more brightly by Vybrant® DyeCycle™ Violet Stain than the chromatin of normal cells.

Ordering information

Product	Apoptotic cell stain	Dead cell stain	Ex/Em	Assays	Cat.No.
Annexin V-Pacific Blue™ conjugate	Annexin V-Pacific Blue™	NA	410/455	100	A35122
Violet Annexin V/Dead Cell Apoptosis Kit with Pacific Blue™ Annexin V/SYTOX® AADvanced™	Annexin V-Pacific Blue™	SYTOX® AADvanced™	410/455 (apoptotic) 546/647 (dead)	50	A35136
Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet/SYTOX® AADvanced™	Vybrant® DyeCycle™ Violet	SYTOX® AADvanced™	369/437 (apoptotic) 546/647 (dead)	200	A35135
Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit	F2N12S	SYTOX® AADvanced™	405/585 (live) 405/530 (apoptotic) 546/647 (dead)	200	A35137
Violet Membrane Permeability/Dead Cell Apoptosis Kit, with PO-PRO™-1 and 7-AAD	PO-PRO™-1	7-AAD	435/455 (apoptotic) 546/647 (dead)	200	V35123

References

Pacific Blue™ and Pacific Orange™ dyes

Telford W et al. (2006) Violet laser diodes in flow cytometry: An update. *Cytometry* 69A:1153–1160.

Melenhorst J et al. (2008) High avidity myeloid leukemia-associated antigen-specific CD8+ T cells preferentially reside in the bone marrow. *Blood* DOI 10.1182/blood-2008-04-151969.

Qdot® nanocrystals

Chattopadhyay PK et al. (2006) Quantum dot semiconductor nanocrystals for immunophenotyping by polychromatic flow cytometry. *Nat Med* 12:972–7.

Chattopadhyay PK et al. (2007) in *Quantum Dots, Applications in Biology.* M. P. Bruchez and C. Z. Hotz, ed. Humana Press, Totowa, NJ. pp. 175–184.

Abrams B and Dubrovsky T (2007) In *Quantum Dots, Applications in Biology.* MP Bruchez and CZ Hotz, eds. Humana Press, Totowa, NJ. pp. 185–206.

Vybrant® DyeCycle™ Violet Stain

Telford W et al. (2007) Side population analysis using a violet-excited cell-permeable DNA binding dye. *Stem Cells* 25:1029–1036.

Multiple dyes, such as amine-reactive viability dyes (ViD), green-fluorescent (GrViD), orange-fluorescent (OrVid), violet-fluorescent (ViViD), UV-fluorescent (UViD)

Perfetto SP et al. (2006) Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. *J Immunol Methods* 313:199–208.

Click-iT™ EdU cell proliferation assay

Buck S et al. (2008) Detection of S-phase cell cycle progression using 5-ethynyl-2′-deoxyuridine incorporation with click chemistry, an alternative to using 5-bromo-2′-deoxyuridine antibodies. *Biotechniques* 44:927–929.

LIVE/DEAD® Fixable Violet Dead Cell Stain Kit

Singh P et al. (2008) Vaccinia virus infection modulates the hematopoietic cell compartments in the bone marrow. *Stem Cells* 26:1009–1016.

Badr G et al. (2008) Early interferon therapy for HCV rescues poly-functional long-lived CD8+ memory T cells. *J Virol* 82:10017–10031.

Charles ED et al. (2008) Clonal expansion of immunoglobulin M+CD27+ B cells in HCV-associated mixed cryoglobulinemia. *Blood* 111:1344–1356.

LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit

Burmeister Y et al. (2008) ICOS controls the pool size of effector-memory and regulatory T cells. *J Immunol* 180:774–782.

SYTOX® Blue Dead Cell Stain

Irion S et al. (2007) Identification and targeting of the ROSA26 locus in human embryonic stem cells. *Nat Biotechnol* 25:1477–1482.

Vybrant® Apoptosis Assay Kits

Vujanovic L et al. (2007) A mycoplasma peptide elicits heteroclitic CD4+ T cell responses against tumor antigen MAGE-A6. *Clin Cancer Res* 13:6796–6806.

Spectral properties and recommended filters for Qdot® nanocrystals and violet laser dyes

Dye/nanocrystal	Excitation wavelength (nm)	Peak emission wavelength (nm)	Recommended filter
Qdot® 565	405*	565	575/26
Qdot® 605	405*	605	605/20
Qdot® 625	405*	625	625/20
Qdot® 655	405*	655	655/20
Qdot® 705	405†	705	720/20
Qdot® 800	405†	800	787/42
Pacific Blue™	410	455	450/50
Pacific Orange™	400	551	585/42
CellTrace™ Violet	391	450	450/50
PO-PRO™-1	435	455	450/50
Calcein violet, AM	400	452	450/50
SYTOX® Blue Dead Cell Stain	444	480	440/40
LIVE/DEAD® Fixable Violet Dead Cell Stain	416	451	450/50
LIVE/DEAD® Fixable Aqua Dead Cell Stain	367	562	525/30
LIVE/DEAD® Fixable Yellow Dead Cell Stain	400	551	585/42
Vybrant® DyeCycle™ Violet Stain	369	437	440/40
FxCycle™ Violet Stain	358	461	450/50

***** Qdot® nanocrystals excite optimally in the UV and 405 nm range but can also be excited with 488 nm lasers. † Qdot® 705 and Qdot® 800 can also be excited with 633 nm lasers.

Fluorophore selection guide for the Attune™ Acoustic Focusing Cytometer

Laser	Excitation laser	Fluorescence channel	Default filter	Filter range (nm)	Fluorophores	Other fluorescent dyes
Violet	405	Blue	450/50	425–475	Pacific Blue™ Alexa Fluor® 405	PO-PRO™-1 DyeCycle™ Violet Fixable Violet Dead Cell Stain CellTrace™ Violet Calcein Violet SYTOX® Blue FxCycle™ Violet
		Green	522/31	507–537		Fixable Aqua Dead Cell Stain
		Yellow	603/48	579–627	Pacific Orange™ Qdot® 605	Fixable Yellow Dead Cell Stain
Blue	488	Green	522/31	507–537	Alexa Fluor® 488 Fluorescein	Calcein Fluo-3/fluo-4 TO-PRO®-1 CFSE GFP JC-1/$DiOC_2(3)$ SYTOX® Green DyeCycle™ Green Rhodamine 123 YO-PRO®-1 Fixable Green Dead Cell Stain
		Yellow	575/26	562–588	PE	PI Fura Red™ DyeCycle™ Orange JC-1/$DiOC_2(3)$ pHrodo™ dye SNARF® (low pH)
		Orange	645LP	>645	PE-Alexa Fluor® 610 PerCP PE-Alexa Fluor® 700 PE-Cy®5.5 TRI-COLOR® PE-Cy®7 PE-Alexa Fluor® 750 Qdot® 655 Qdot® 705 Qdot® 800	PI JC-1/$DiOC_2(3)$ Fixable Red Dead Cell Stain 7-AAD SNARF® (high pH) SYTOX® AADvanced™ DyeCycle™ Ruby

Notes:

For more than 30 years, **MOLECULAR PROBES®** has developed fluorescence technology that enables uniquely powerful labeling and detection solutions for cellular analysis. Their innovations have led to proprietary technologies that significantly advance assay sensitivity and specificity, opening research avenues that were previously impractical or impossible.

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