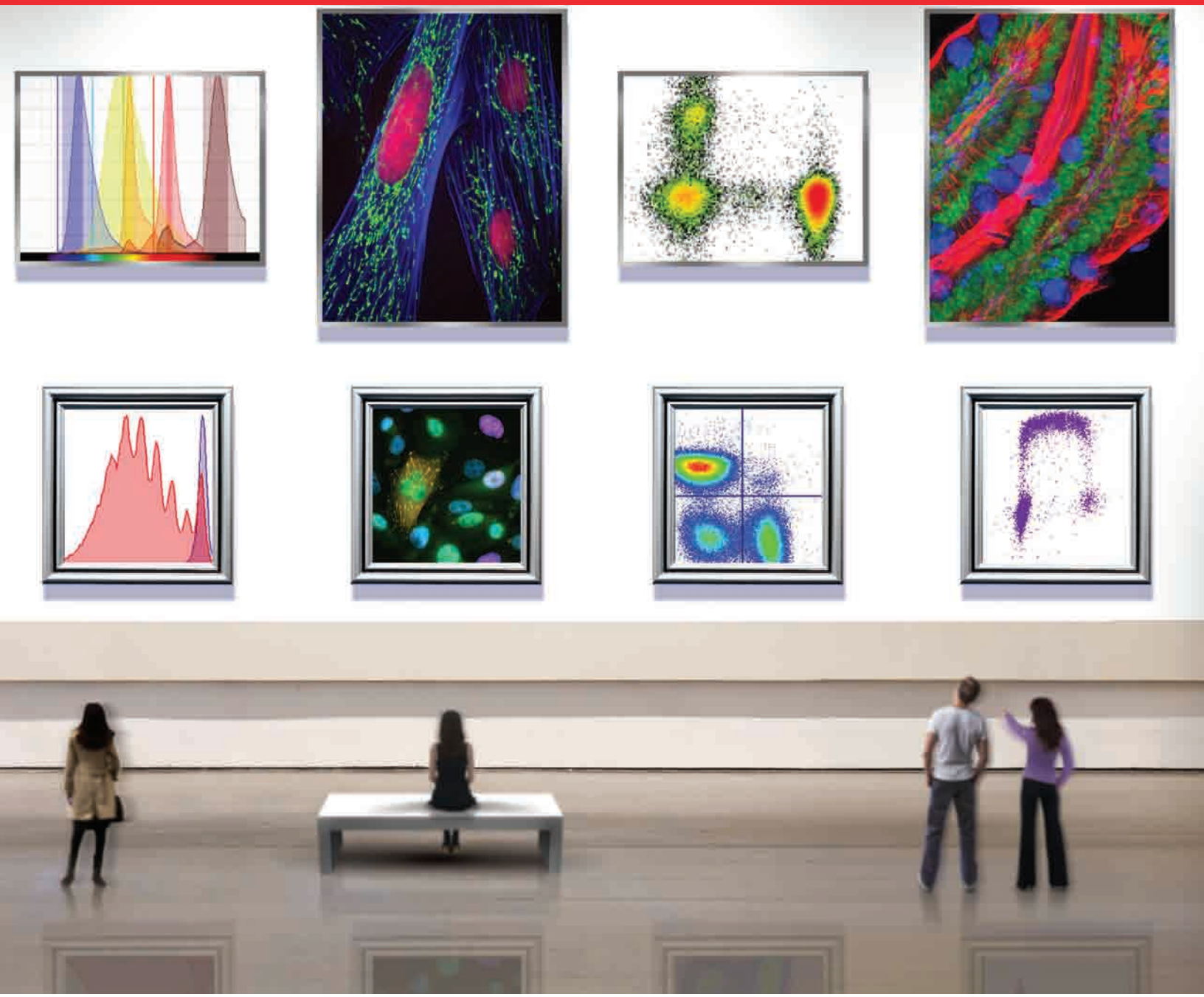
invitrogen



Molecular Probes flow cytometry

Products and resource guide

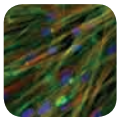
ThermoFisher SCIENTIFIC

Resources

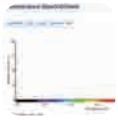
Reference guide

The Molecular Probes™ Handbook, 11th Edition
The most complete reference on fluorescent labeling and detection available, this resource features extensive references and technical notes, and contains over 3,000 technology solutions representing a wide range of biomolecular labeling and detection reagents. Download the chapters, request a free copy* and access the online version of the Handbook at **thermofisher.com/handbook**

Online tools



Panel design tool
Use this tool to build out your multicolor flow cytometry antibody panel. Learn more at **thermofisher.com/flowpanel**



Fluorescence SpectraViewer
Plot up to 14 fluorophores on a single graph, which you can then print or save for later. **thermofisher.com/spectraviewer**

Flow cytometry resources center
Search for protocols, tutorials, application notes, fluorophore and product selection guides, literature, and many other technical resources in a single place. **thermofisher.com/flowresources**

Flow cytometers and accessories
The next step in the evolution of cell-by-cell analysis is here. The Invitrogen™ Attune™ NxT Cytometer enables you to optimize performance and throughput without sacrificing sensitivity or accuracy. **thermofisher.com/attune**

Stay in the know—news and updates

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Our award-winning print and online magazine, highlights the latest breakthroughs from our scientists, featuring new technologies and products.

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Learn and connect

Molecular Probes School of Fluorescence
Learn about the fundamentals of fluorescence, imaging and flow cytometry. Find protocols, troubleshooting guides and more at **thermofisher.com/mpsf**

Mobile apps for research on the go

Flow cytometry guide and protocols
Get access to our reagent selection guides for selected applications and protocols, and troubleshoot on the go. Also enjoy a built-in protocol timer and alarm for timed steps.

Fluorescence SpectraViewer
Plot and compare spectra, check spectral compatibility for fluorophores, and email the configuration to yourself in a clear, printable format.

DailyCalcs science calculator
Easily calculate molarity, dilution, molecular weight, and more.

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Molecular Probes flow cytometry

Products and resource guide

Flow cytometry workflow

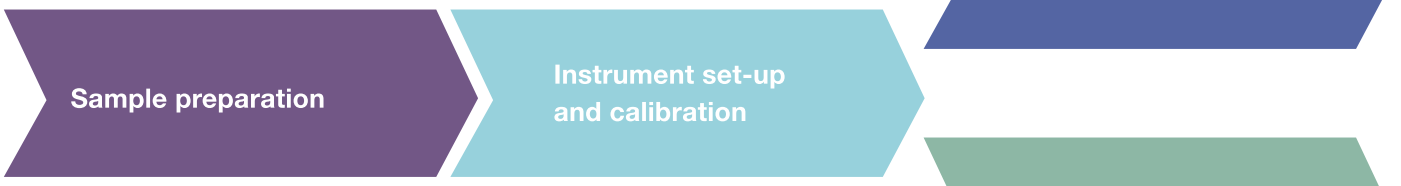
Reagents

The Invitrogen™ Molecular Probes™ flow cytometry products and resource guide presents an overview of primary antibody conjugates, cell function assays, and other tools optimized for flow cytometry by our scientists. We have been at the forefront of invention and development of fluorescent probes for over 40 years, and this guide features some of the most useful flow cytometry tools available anywhere. For the probes and assays you need, available in a full spectrum of fluorescent colors, look to Molecular Probes flow cytometry products first.

Attune NxT flow cytometer

The Attune NxT Flow Cytometer is a benchtop analyzer that uses a revolutionary technology—acoustic focusing—to align cells or particles prior to the laser interrogation points. Now with the flexibility to create a customized 4-laser, 14-color system, the Attune NxT flow cytometer is designed to accommodate a variety of experimental protocols and any lab budget.

See page 32 for more information.



Visit **thermofisher.com/flow-cytometry** for more information on Molecular Probes flow cytometry products, the Attune NxT flow cytometer and flow cytometry resources.

Sample preparation

Quality data require quality starting material. Invitrogen™ Molecular Probes™ sample preparation reagents, which include reagents for blood cell preservation, red blood cell lysis, and sample fixation and permeabilization, are designed to help you achieve the best possible results. Learn more about these products and get protocols at **thermofisher.com/flow-sample**

Cell preservation

TransFix Reagent

Invitrogen™ TransFix™ Cellular Antigen Stabilizing Reagent (Cat. No. FIX2, FIX20, FIX100) is a whole-blood preservative that stabilizes leukocytes and preserves antigenic sites for future cellular analysis. The reagent allows simple batch processing of multiple samples to optimize your laboratory workflow. Additionally, blood that has been stabilized with TransFix Reagent can be easily transported to other sites for analysis.

Cell lysis

Cal-Lyse whole blood lysing solution

The Invitrogen™ Cal-Lyse™ premixed lysing solution (Cat. No. GAS010, GAS010S100) is specifically formulated to lyse red blood cells following monoclonal antibody staining of whole blood or bone marrow. Treatment with this reagent leads to both the lysis of red blood cells and the fixation of white cells. Treatment does not affect fluorophore-labeled antibodies bound to leukocytes, and leaves morphological light-scattering characteristics of leukocytes intact. Cal-Lyse reagent can be used for either “no-wash” or “wash” staining procedures.

Fixative-free lysing solution High-Yield Lyse

Invitrogen™ High-Yield Lyse (Cat. No. HYL250) is a premixed, fixative-free erythrocyte lysing solution that can be used to eliminate red cells from whole blood for flow cytometric analysis, with minimal loss of rare blood cell populations. Using this reagent, erythrocyte lysis is performed immediately following staining of the blood samples with monoclonal antibodies; therefore, there is no need for a wash step.

Fixation and permeabilization

FIX & PERM reagents and kits

- Compatible with analysis of most cellular antigens
- No effect on cellular morphological scatter
- Reduced background staining
- Proven protocols

The Invitrogen™ FIX & PERM™ Cell Permeabilization Kit (Cat. No. GAS003, GAS004) consists of matched Fixation Medium (Medium A) and Permeabilization Medium (Medium B) for simultaneous analysis of intracellular and cell-surface antigens in the same cell population (Figure 1). This procedure facilitates antibody access to intracellular structures and leaves the morphological light-scattering characteristics of the cells intact. These formulations reduce background staining and allow simultaneous addition of permeabilization medium and fluorophore-labeled antibodies. The Fixation Medium (Cat. No. GAS001S100) and Permeabilization Medium (Cat. No. GAS002S100) are available separately as well.

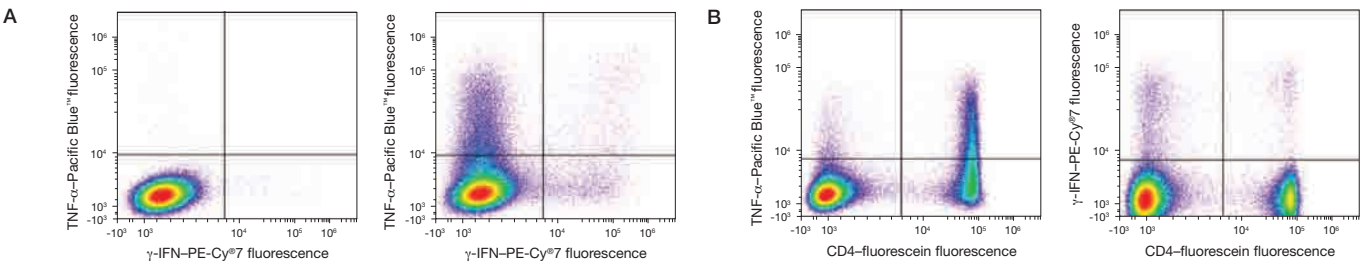


Figure 1. Use of FIX & PERM Cell Permeabilization Kit for simultaneous surface antigen and intracellular antigen staining. C57BL/6 splenocytes were left unstimulated or stimulated for 5 hours with phorbol myristate acetate (PMA) and ionomycin in the presence of brefeldin A. Cells were then surface-stained with fluorescein (FITC)-conjugated anti-mouse CD4 antibody (Cat. No. MCD0401). This step was followed by fixation and permeabilization of the sample using the FIX & PERM Cell Permeabilization Kit (Cat. No. GAS003, GAS004). Intracellular staining was performed during the permeabilization step using Invitrogen™ PE-Cy®7 tandem–conjugated anti-mouse γ-interferon (γ-IFN) antibody (Cat. No. A18713) and Invitrogen™ Pacific Blue™ dye–conjugated anti-mouse tumor necrosis factor α (TNF-α) antibody (Cat. No. RM90128). Data were collected using the Attune flow cytometer (blue/violet) with 488 nm excitation and a 530/30 nm bandpass emission filter to detect FITC fluorescence and a 640 nm longpass filter to detect PE-Cy7 tandem fluorescence. Pacific Blue conjugate fluorescence was detected using 405 nm excitation and a 450/40 nm bandpass emission filter. (**A**) γ-IFN and TNF-α antibody co-staining of total mouse splenocytes, gated on lymphocytes, that were left unstimulated (**left**) or stimulated (**right**) with PMA and ionomycin in the presence of brefeldin A. (**B**) $CD4^+$ T cell expression of TNF-α (**left**) and γ-IFN (**right**) after stimulation as described above.

Dynabeads cell isolation

- Viable and functional—products for positive isolation, negative isolation, cell activation, and depletion
- Gentle—column-free separation and inert bead surfaces translate to gentler handling of your cells and help reduce the risk of contaminants in the preparations
- High yields—tube-based separation allows you to achieve excellent recovery of cells

When cells are removed from their natural environment, there is a risk that experimental procedures will negatively impact cell phenotype and function. Choosing the right cell separation method is therefore critical to downstream experiments. Invitrogen™ Dynabeads™ magnetic beads are superparamagnetic, monosized polymer beads coated with a thin, inert polymer shell to encase the magnetic material (Figure 2). This design helps to reduce the risk that any unwanted material such as iron is left in the sample after separation. In addition, since the cells are never bound to the beads (negative-isolation and depletion beads) or are released from the beads after the gentle magnetic separation (positive-isolation beads), the final cell sample is of high purity and viability, with no process-derived remnants that could affect the results (Figure 3).

Human cell isolation

Dynabeads magnetic beads enable gentle tube-based isolation of human cells directly from whole blood, mononuclear cells, buffy coat, bone marrow, or tissue samples for any downstream assay, including flow cytometry (Figure 4). Dynabeads products are available for isolating human T cells, B cells, stem cells, NK cells, monocytes, dendritic cells, endothelial cells, tumor cells, leukocytes, and granulocytes.

Isolation options include:

- Positive isolation and cell release
- Negative isolation, resulting in untouched cells
- Depletion of unwanted cell types or positive cell isolation for molecular applications
- Isolation of cells using your own antibody

If you can't find a ready-to-use product for human cell isolation, we have a range of Dynabeads products that can be combined with an antibody of your choice to create a tailored cell isolation tool that include: streptavidin beads, secondary antibody–coated beads, and surface-activated beads. Learn more at **thermofisher.com/humancellisolation**

Figure 2. Dynabeads products are uniform spherical beads with highly defined and consistent product characteristics, which helps ensure that you get truly reliable and reproducible results.

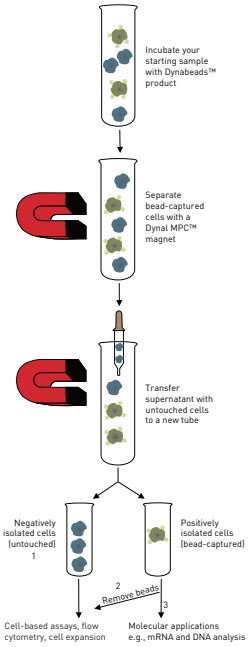


Figure 3. Workflow diagram for using Dynabeads beads for positive or negative tube-based cell isolation.

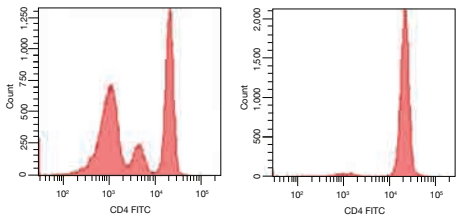


Figure 4. Purity of human CD4⁺ T cells. Purity before (**left**) and after (**right**) negative isolation from peripheral blood mononuclear cells using the Invitrogen™ Dynabeads™ Untouched™ Human CD4 T Cells Kit (Cat. No. 11352D).

Mouse cell isolation

Dynabeads products offer gentle tube-based isolation of mouse cells directly from whole blood, spleen, lymph node, or thymus for any downstream assay, including flow cytometry (Figure 5). Dynabeads products are available for isolating mouse T cells, B cells, NK cells, and dendritic cells.

Isolation options include:

- Positive isolation and cell release
- Negative isolation of untouched cells
- Depletion of unwanted cell types or positive cell isolation for molecular applications

If you can't find a ready-to-use product for mouse cell isolation, we have a range of Dynabeads products that can be combined with an antibody of your choice to create a tailored cell isolation tool: streptavidin beads, secondary antibody–coated beads, and surface activated beads. Learn more at **thermofisher.com/mousecellisolation**

Find more information online

If you go to **thermofisher.com/cellisolation**, you can find information about our entire line of cell isolation products, and you'll also have access to:

- Selection guides for choosing the correct cell isolation product
- Protocols for sample preparation and strategies for cell isolation
- Data showing the performance of our cell isolation products versus other commercially available products
- Help in choosing the correct magnets for your tubes or plates
- Links to videos, brochures, and application notes and to references that cite the use of Dynabeads products

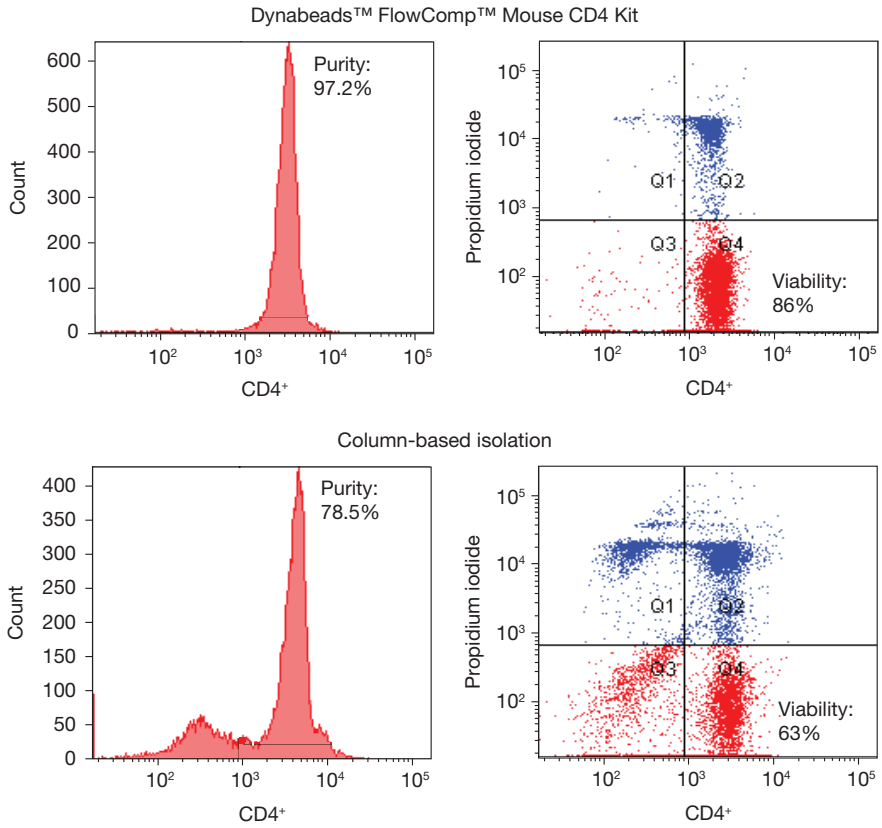


Figure 5. Isolation of CD4⁺ T cells from mouse spleen cells. Cell isolation using the Invitrogen™ Dynabeads™ FlowComp™ Mouse CD4 Kit (Cat. No. 11461D) (**top**) results in substantially higher purity (97%) and viability (86%) than column-based (**bottom**) positive cell isolation (yielding purity and viability of 78% and 63%, respectively).

Sample preparation product list

Section name	Product name	Species	Target cell	Regulatory status	Size	Cat. No.
Research Use Only (RUO)						
Cell preservation	TransFix Cellular Antigen Stabilizing Reagent	NA	NA	RUO*	2 mL	FIX2
					20 mL	FIX20
					100 mL	FIX100
Dynabeads cell isolation	Dynabeads FlowComp Human CD4 Kit	Human	T cells	RUO	3 mL	11361D
	Dynabeads CD4 Positive Isolation Kit	Human	T cells	RUO	5 mL	11331D
	Dynabeads Untouched Human CD4 T Cells Kit	Human	T cells	RUO	2 mL	11352D
	Dynabeads FlowComp Human CD8 Kit	Human	T cells	RUO	3 mL	11362D
	Dynabeads CD8 Positive Isolation Kit	Human	T cells	RUO	5 mL	11333D
	Dynabeads Untouched Human CD8 T Cells Kit	Human	T cells	RUO	1 kit	11348D
	Dynabeads FlowComp Mouse CD4 Kit	Mouse	T cells	RUO	3 mL	11461D
	DETACHaBEAD Mouse CD4 Kit	Mouse	T cells	RUO	5 mL	12406D
	Dynabeads Untouched Mouse CD4 Cells Kit	Mouse	T cells	RUO	2 x 10 mL	11415D
	Dynabeads FlowComp Mouse CD8 Kit	Mouse	T cells	RUO	3 mL	11462D
	Dynabeads Untouched Mouse CD8 Cells Kit	Mouse	T cells	RUO	2 x 10 mL	11417D
General Purpose Reagent (GPR)						
Fixation and permeabilization	FIX & PERM Cell Permeabilization Kit	NA	NA	GPR**	50 assays	GAS003
					200 assays	GAS004
	Fixation Medium (Medium A)	NA	NA	GPR	100 mL	GAS001S100
	Permeabilization Medium (Medium B)	NA	NA	GPR	100 mL	GAS002S100
In Vitro Diagnostic (IVD)						
Red blood cell lysis	Cal-Lyse Whole Blood Lysing Solution	NA	NA	IVD†	25 mL	GAS010
					100 mL	GAS010S100
	High-Yield Lyse	NA	NA	IVD	500 mL	HYL250

NA, not applicable.
*RUO: For Research Use Only. Not for use in diagnostic procedures, unless otherwise indicated.
**GPR: For Laboratory Use.
†IVD: For In Vitro Diagnostic Use.

Instrument set-up and calibration

- Helps to ensure the reliability of optimal daily instrument performance
- Leads to minimal variation for consistent data acquisition
- Compatible with any instrument

Flow cytometers are designed to perform quantitative measurements on individual cells and other particles with high precision, speed, and accuracy. As with all high-performance instrumentation, flow cytometers must be calibrated frequently to ensure accuracy and reliability. The stability, uniformity, and reproducibility of Invitrogen™ Molecular Probes™ microsphere products make them excellent tools for flow cytometer instrument set-up and calibration. Learn more about these Molecular Probes products at **thermofisher.com/flow-standards**

Alignment

AlignFlow Cytometry Alignment Beads

Invitrogen™ AlignFlow™ Flow Cytometry Alignment Beads are reliable references for aligning, focusing, and calibrating flow cytometers. These fluorescently stained polystyrene microspheres are highly uniform with respect to size and fluorescence intensity (Figure 6), and are designed to approximately replicate the size, emission wavelength, and intensity of biological samples. Because the dyes are contained inside the microsphere's matrix, instead of on the bead's surface, AlignFlow beads have excellent photochemical and physical stability, providing reliable reference signals for instrument set-up. The fluorescent dyes have been carefully selected for optimal excitation by laser sources commonly used in flow cytometry. The AlignFlow beads are available in three versions: for 350–370 nm excitation with UV lasers (Cat. No. A16502, A16505), for 488 nm excitation with blue lasers (Cat. No. A16500, A16503), and for 633 nm excitation with red lasers (Cat. No. A16501, A16504). Each version is available in two bead sizes: 2.5 µm diameter and 6.0 µm diameter.

Size calibration

Flow Cytometry Size Calibration Kit

The Invitrogen™ Flow Cytometry Size Calibration Kit (Cat. No. F13838) provides a set of nonfluorescent microsphere suspensions to serve as reliable size references for cytometry users. The kit contains six suspensions of unstained polystyrene microspheres, each with a known diameter, determined by transmission electron microscopy. The size of cells in an experimental sample can be estimated by comparing the forward scatter (FSC) signals with those of the reference microspheres. The microspheres function as reproducible size markers (Figure 7) and can be intermixed with the experimental sample or used in parallel runs.

Flow Cytometry Sub-micron Particle Size Reference Kit

The Invitrogen™ Flow Cytometry Sub-micron Particle Size Reference Kit (Cat. No. F13839) provides a set of green-fluorescent microsphere suspensions to serve as reliable size references for flow cytometry users. The kit contains six suspensions of polystyrene microspheres, each with a known diameter as determined by transmission electron microscopy. The excitation and emission profile of all the beads is similar to Invitrogen™ Alexa Fluor™ 488– or FITC-stained cells (excitation and emission maxima are 505 nm and 515 nm, respectively).

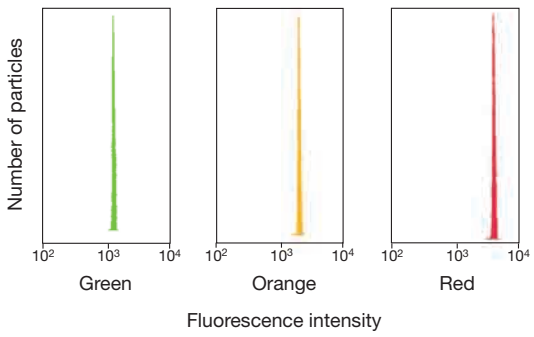


Figure 6. AlignFlow beads excited at 488 nm by an argon-ion laser and monitored in three emission channels. Broad fluorescence emission is detected in all three channels. Note the exceptionally small variation in fluorescence intensity of the beads. Data contributed by Carleton Stewart, Rosewell Park Cancer Institute.

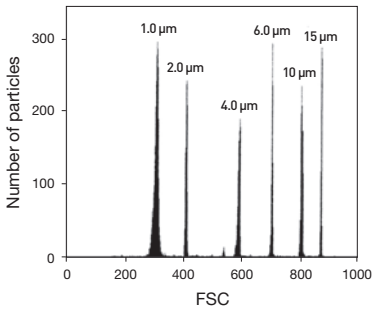


Figure 7. Flow Cytometry Size Calibration Kit. Histogram analysis of the forward scatter intensity (FSC) log channel values of the six polystyrene microsphere samples supplied in the Flow Cytometry Size Calibration Kit (Cat. No. F13838) is shown. FSC measurements were performed on a Becton Dickinson FACScan™ flow cytometer using excitation at 488 nm.

The size (or size range) of bioparticles in an experimental sample can be estimated by comparing their FSC with those of the reference microspheres (Figure 8). The microspheres in each component function as reproducible size markers and can be used individually (one size), premixed (two to six sizes), intermixed with the experimental sample, or in parallel runs.

This kit can be used to verify instrument performance and to establish parameters that are suitable for analyzing sub-micron particles. For example, the kit can be used to check:

- Resolution limit and dynamic range of particle size measurement
- Sensitivity of forward and side scatter photomultiplier tubes
- Level of instrument baseline noise
- Laser and optical alignment and stability
- Stability of the fluidics system

Cell sorting set-up

Cell Sorting Set-Up Beads

The Invitrogen™ Cell Sorting Set-Up Beads (Cat. No. C16506, C16507, C16508, C16509) are reliable standards for the set-up and calibration of flow cytometry sorter instruments. The beads have a diameter of 6 µm (±10%), and thus approximate the size, emission wavelength, and intensity of many biological samples. Consequently, the beads can be used to check cell sorter settings such as drop delay and efficiency (cell loss during sorting). The beads can also be used to calibrate a flow cytometer's laser source, optics, and stream flow without wasting valuable and sensitive experimental material.

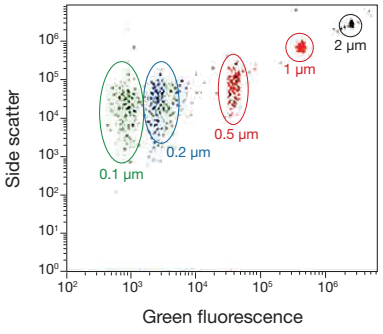


Figure 8. Flow Cytometry Sub-micron Particle Size Reference Kit. Signals from (the kit has six particle sizes; only five were used here) five different-sized particles of the Flow Cytometry Sub-micron Particle Size Reference Kit (Cat. No. F13839) were acquired using 488 nm excitation and a 530/30 nm bandpass (BP) emission filter on the Attune flow cytometer. The diameters of the five different green-fluorescent microspheres are identified on a plot of particle fluorescence versus side scatter.

Compensation

In a perfect world, the fluorescence emission profile for each individual fluorophore would be a very intense, narrow peak, well-separated from all other emission peaks. In reality, organic dyes and fluorescent proteins have broad emission peaks. An example of the overlap of two commonly used fluorophores is shown in Figure 9, which features the emission profiles of Alexa Fluor 488 dye and R-phycoerythrin (R-PE). For proper interpretation of the data collected, it is important to know that the fluorescent signal being recorded for Alexa Fluor 488 dye is, in fact, coming from Alexa Fluor 488 dye and not from R-PE, which happens to emit some light in the same wavelength range. To accurately record the fluorescence signal for a given fluorophore, it is important to correct for the emission signal of all dyes, and this correction is called compensation.

We offer two types of compensation kits (Table 1). One type, the Invitrogen™ AbC™ bead kits, is designed for compensation of dyes in immunophenotyping experiments using fluorescently-labeled antibodies. The second type of compensation kit, the Invitrogen™ ArC™ Amine Reactive Compensation Bead Kit, is designed for use with cell viability assays that use amine reactive dyes, such as the Invitrogen™ LIVE/DEAD™ Fixable Dead Cell Stain Kits (see "Cell viability" on page 20 for more details on these assays).

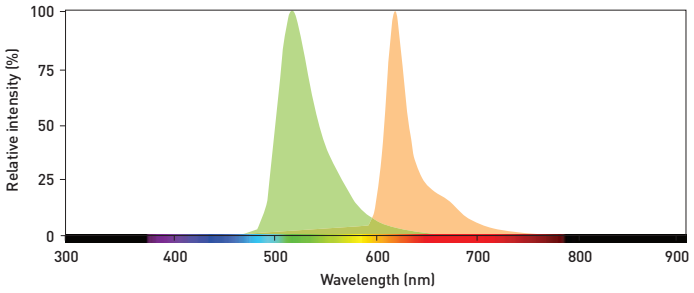


Figure 9. Emission profiles of Alexa Fluor 488 dye (green curve) and R-PE (orange curve).

Table 1. Compensation kit selection guide.

Product name	Assay type	Positive bead	Negative bead	Cat. No.
AbC Total Antibody Compensation Bead Kit	Immunophenotyping	Hamster, mouse, rabbit, and rat antibodies*	No binding capacity	A10497, A10513
AbC Anti-Mouse Bead Kit	Immunophenotyping	Mouse monoclonal antibodies*	No binding capacity	A10344
AbC Anti-Rat/Hamster Bead Kit	Immunophenotyping	Rat and hamster monoclonal antibodies*	No binding capacity	A10389
ArC Amine Reactive Compensation Bead Kit	Cell viability assay	Amine reactive dyes and LIVE/DEAD Fixable Dead Cell Stains	No amine reactive capacity	A10346

*AbC capture beads bind all isotypes.
All products in this table are RUO, For Research Use Only. Not for use in diagnostic procedures.

AbC compensation bead kits

- An alternative to using precious samples for setting flow cytometry compensation
- Highest reactivity to different subclasses of mouse, rat, and hamster immunoglobulin
- Fast and simple bead-based flow cytometry compensation
- Removal of inconsistencies due to variations in antigen expression

AbC bead kits provide a consistent, accurate, and simple-to-use technique for the setting of flow cytometry compensation when using 1) fluorophore-conjugated hamster, mouse, rabbit, or rat antibodies (AbC Total Antibody Compensation Bead Kit, Cat. No. A10497, A10513; Figure 10); 2) using fluorophore-conjugated mouse antibodies (AbC Anti-Mouse Bead Kit, Cat. No. A10344, Figure 11); or 3) using fluorophore-conjugated rat or hamster antibodies (AbC Anti-Rat/Hamster Bead Kit, Cat. No. A10389). All three kits contain two types of specially modified polystyrene microspheres: 1) AbC capture beads (also called positive beads), which bind all isotypes of the specific immunoglobulin, and 2) negative beads, which have no antibody binding capacity. After incubation with a fluorophore-conjugated primary antibody (hamster, mouse, rat, or rabbit, depending on the kit used), the two components provide distinct positive and negative populations of beads that can be used to set compensation (Figure 11).

ArC Amine Reactive Compensation Bead Kit

- Eliminates the hassle of heat-treating cells
- Optimized for all LIVE/DEAD Fixable Dead Cell Stain kits
- Fast and simple bead-based flow cytometry compensation
- An alternative to using precious samples for setting compensation
- Enables accurate and consistent results

The ArC Amine Reactive Compensation Bead Kit (Cat. No. A10346) provides a consistent, accurate, and simple-to-use technique for the setting of flow cytometry compensation when using any of the LIVE/DEAD Fixable Dead Cell Stain kits or when using any amine reactive dye. LIVE/DEAD Fixable Dead Cell Stain kits (and amine reactive dyes) can be used to evaluate mammalian cell viability based on the fact that the dye reacts with cellular amines. The reactive dye can enter the cell via compromised membranes of necrotic cells and react with free amines in the interior and on the surface of the cell, resulting in intense fluorescent staining. In contrast, only the cell-surface amines of viable cells are available to react with the dye, resulting in relatively dim staining. The difference in fluorescence intensity between the live and dead cell populations is typically greater than 50-fold.

The ArC Amine Reactive Compensation Bead Kit includes two types of specially modified polystyrene microspheres to allow easy compensation of the LIVE/DEAD Fixable Dead Cell Stains: the ArC reactive beads (Component A), which bind any of the amine reactive dyes, and the ArC negative beads (Component B), which have no reactivity. After incubation with any amine reactive dye, the two kit components provide

distinct positive and negative populations of beads that can be used to set compensation (Figure 12). The ArC Amine Reactive Compensation Bead Kit can be combined with the AbC Anti-Mouse Bead Kit for use with fluorophore conjugated mouse antibodies, allowing even more consistent and accurate compensation for multicolor immunophenotyping experiments that also incorporate a LIVE/DEAD Fixable Dead Cell Stain.

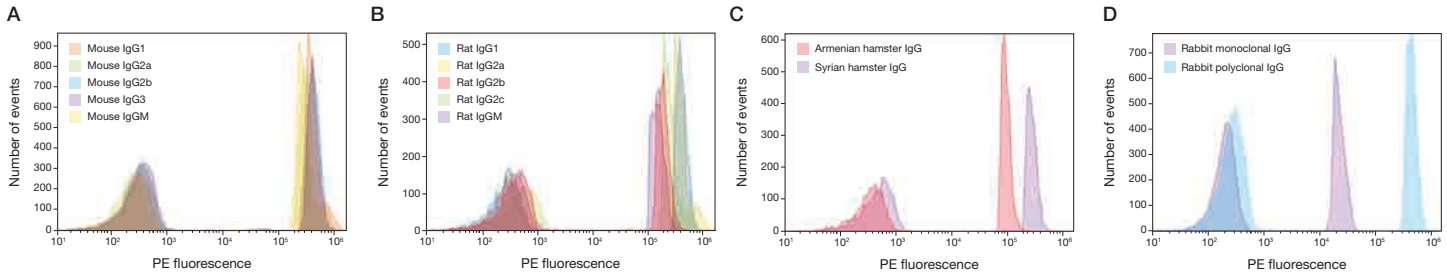


Figure 10. Histograms showing the staining of the AbC Total Antibody Compensation Bead Kit. Signal separation of the positive capture beads for mouse (**A**), rat (**B**), and hamster (**C**) monoclonal antibodies, and rabbit (**D**) monoclonal and polyclonal antibodies. Beads were labeled with an optimized amount of each PE antibody conjugate and analyzed on an Attune cytometer using 488 nm excitation and a 574/26 nm bandpass filter.

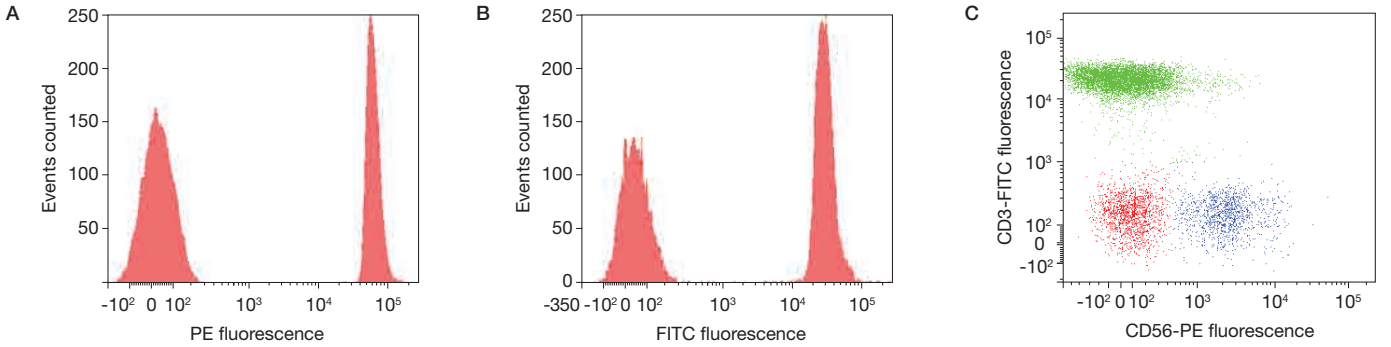


Figure 11. Compensation using the AbC Anti-Mouse Bead Kit. (**A**) Phycoerythrin (PE)-conjugated mouse anti-human CD56 antibody (Cat. No. MHCD5604) was used with AbC capture beads for a positive signal and with negative beads for a negative signal. (**B**) FITC-conjugated mouse anti-human CD3 antibody (Cat. No. MHCD03014) was used with AbC capture beads for a positive signal and with negative beads for a negative signal. (**C**) Dual-parameter plot showing gated human lymphocytes labeled with PE–conjugated mouse anti-human CD56 and FITC-conjugated mouse anti-human CD3 antibodies after compensation was performed with the AbC Anti-Mouse Bead Kit (Cat. No. A10344).

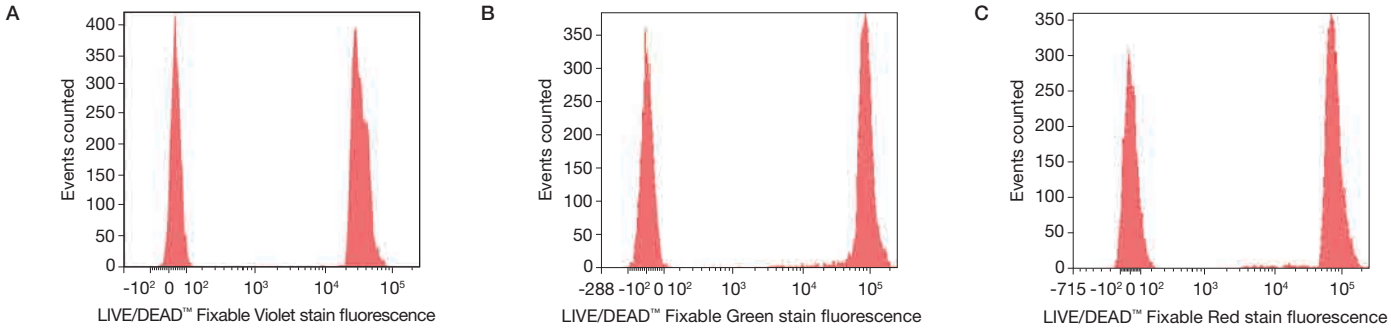


Figure 12. Staining profile of the ArC Amine Reactive Compensation Bead Kit components with 3 LIVE/DEAD Fixable Dead Cell Stain kits. (**A**) LIVE/DEAD Fixable Violet dye stained beads (Cat. No. L34955) were analyzed with 405 nm excitation, emission was collected with a 450/50 nm bandpass filter. (**B**) LIVE/DEAD Fixable Green dye stained beads (Cat. No. L23101) were analyzed with 488 nm excitation, emission was collected with a 525/50 nm bandpass filter. (**C**) LIVE/DEAD Fixable Far Red dye stained beads (Cat. No. L10120) were analyzed using 633 nm excitation, emission was collected with a 660/20 nm bandpass filter.

Table 2. Absolute counting beads selection guide.

Product name	Sample type	Bead size	Excitation (nm)	Emission max (nm)	Parameters measured	Regulatory status*	Cat. No.
CountBright Absolute Counting Beads	Any type	7 µm	UV to 635	385 to 800	Number of cells	RUO	C36950
AccuCheck Counting Beads	Whole blood	Bead A: 6.40 µm Bead B: 6.36 µm	Bead A: 488 Bead B: 635	Bead A: 575–585 Bead B: 660–680	Number of cells and accuracy of pipetting	RUO	PCB100

*For Research Use Only. Not for use in diagnostic procedures, unless otherwise indicated.

Absolute cell counting

Flow cytometry provides a rapid method to quantify cell characteristics. However, most flow cytometers cannot directly provide the cell concentration or absolute count of cells in a sample. Absolute cell counts have been widely used in quantifying cell populations and disease progression, including in studies of stem cells. Absolute cell counts are generally obtained either by combining a separate cell concentration determination from a hematology analyzer with flow cytometric population data (multiple platform testing) or by adding an internal microsphere counting standard to the flow cytometric sample (single platform testing). The single platform method is preferred as it is technically less complicated and it avoids interlaboratory variation and underestimations, making it more accurate than multiple platform testing.

We offer two products for cell counting—Invitrogen™ CountBright™ Absolute Counting Beads and Invitrogen™ AccuCheck™ Counting Beads. See Table 2 to determine which kit is right for your experiment.

CountBright Absolute Counting Beads

- Compatible with every commercially available flow cytometer because they are loaded with a wide breadth of fluorochromes
- Easy-to-use protocol that works with multiple cell types including lysed/no-wash whole blood
- More reliable than multiple platform testing

CountBright Absolute Counting Beads (Cat. No. C36950) are a calibrated suspension of microspheres that are brightly fluorescent across a wide range of excitation and emission wavelengths (UV to 635 nm excitation and 385 to 800 nm emission) and contain a known concentration of microspheres.

For absolute counts, a specific volume of the microsphere suspension is added to a specific volume of sample, so that the ratio of sample volume to microsphere volume is known. The volume of sample analyzed can be calculated from the number of microsphere events, and can be used with cell events to determine cell concentration (Figure 13). In general, at least 1,000 bead events should be acquired to assure a statistically significant determination of sample volume.

CountBright Absolute Counting Beads can be used with any sample type, including lysed/no-wash whole blood. The microspheres in the reagents are approximately 7 µm in diameter and have settling properties similar to lymphocytes. Sample preparation steps that can lead to cell or microsphere loss, such as washes, should be avoided. CountBright beads can be used with either a scatter or fluorescence threshold. When using a scatter threshold, the microsphere signal should be above the threshold. The microspheres can be gated by a single parameter, but a combination of parameters can be used to resolve microspheres from cells and other events.

AccuCheck Counting Beads

- Internal control using ratio of two different color beads indicates pipetting accuracy
- Single platform is preferred over multiple platform testing, to enable consistency in results
- Easy to validate with most immunophenotyping experiments

AccuCheck Counting Beads (Cat. No. PCB100) are an efficient single platform method for absolute cell counting that combines the advantages of direct flow cytometric immunophenotyping with the use of two different fluorescent beads (A and B beads). These two fluorospheres are used as a double internal standard for blood volume calculation.

A known volume of AccuCheck Counting Beads is added to the same known volume of stained blood in a lysed/no-wash technique. The beads are counted along with cells. Because the concentration of beads is known, the number of cells per microliter (the absolute count) is obtained by relating the number of cells counted to the total number of fluorescent bead events. The cell number is then multiplied by the number of total fluorospheres per unit of volume. As the AccuCheck Counting Beads system contains two different fluorospheres in a known proportion, the accuracy of the assay pipetting can be verified using the proportion of both types of beads.

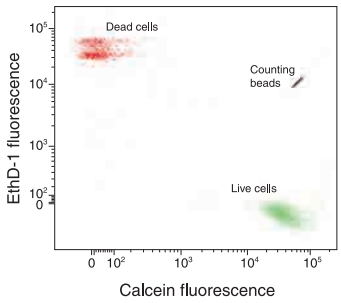


Figure 13. CountBright Absolute Counting Beads. A mixture of live and heat-killed Jurkat cells were treated with reagents in the LIVE/DEAD Viability/ Cytotoxicity Kit (Cat. No. L3224). CountBright Absolute Counting Beads (Cat. No. C36950) were added to the sample, which was then analyzed by flow cytometry using 488 nm excitation. Calcein fluorescence was collected with a 530/30 nm bandpass filter and ethidium homodimer-1 (EthD-1) fluorescence was collected with a 610 nm longpass filter. The data show clear separation of live and dead cells, as well as separation of the counting beads.

Instrument set-up and calibration product list.

Section	Product name	Laser type	Ex/Em*	Regulatory status†	Size	Cat. No.
Alignment	AlignFlow Flow Cytometry Alignment Beads for UV Lasers, 2.5 µm	UV	350–370/ 400–470	RUO	3 mL	A16502
	AlignFlow Flow Cytometry Alignment Beads for UV Lasers, 6.0 µm					A16505
	AlignFlow Flow Cytometry Alignment Beads for Blue Lasers, 2.5 µm	Blue	488/515–660	RUO	3 mL	A16500
	AlignFlow Flow Cytometry Alignment Beads for Blue Lasers, 6.0 µm					A16503
	AlignFlow Flow Cytometry Alignment Beads for Red Lasers, 2.5 µm	Red	633/645–680	RUO	3 mL	A16501
	AlignFlow Flow Cytometry Alignment Beads for Red Lasers, 6.0 µm					A16504
Size calibration	Flow Cytometry Size Calibration Kit (nonfluorescent microspheres)	NA	NA	RUO	1 kit	F13838
	Flow Cytometry Sub-micron Particle Size Reference Kit	Blue	505/515	RUO	1 kit	F13839
Cell sorting set-up	Cell Sorting Set-Up Beads for UV Lasers	UV	350–375/460	RUO	3 mL	C16506
	Cell Sorting Set-Up Beads for Blue Lasers	Blue	488/515	RUO	3 mL	C16508
	Cell Sorting Set-Up Beads for Green-Yellow Lasers	Green-yellow	532, 561/575	RUO	3 mL	C16509
	Cell Sorting Set-Up Beads for Red Lasers	Red	633/680	RUO	3 mL	C16507
Compensation	AbC Total Antibody Compensation Bead Kit	NA	NA	RUO	100 tests 25 tests	A10497 A10513
	AbC Anti-Mouse Bead Kit	NA	NA	RUO	1 kit	A10344
	AbC Anti-Rat/Hamster Bead Kit	NA	NA	RUO	1 kit	A10389
	ArC Amine Reactive Compensation Bead Kit	NA	NA	RUO	1 kit	A10346
Absolute cell counting	AccuCheck Counting Beads	Blue/red	488/575-585 (Bead A) 635/660–680 (Bead B)	RUO	10 mL	PCB100
	CountBright Absolute Counting Beads	UV to red	UV to 635/385 to 800	RUO	5 mL	C36950

NA, not applicable.
*Excitation and emission maximum wavelengths, in nm.
†RUO, For Research Use Only. Not for use in diagnostic procedures.

Antigen detection

Primary antibodies

Broad range of dye, protein, and Invitrogen™ Qdot™ antibody conjugates

- Wide breadth of specificities for numerous types of applications
- Quality products for clinical and research applications
- Referenced in over 20,000 journal citations

We offer a diverse array of highly specific primary antibodies against targets studied in both basic and clinical research settings. These antibodies are validated for flow cytometry and include specificities for CD markers, cytokines, chemokines, and growth factors, as well as antibodies with utility for oncology, immunology, cell signaling, apoptosis, proliferation, and stem cell research. From subset identification of heterologous cell populations to rare-event detection, multicolor flow cytometry with Molecular Probes fluorescently conjugated primary antibodies can help answer complex cell biology questions—in less time and with less sample than other approaches. Search for your primary antibody conjugate at **thermofisher.com/flow-searchantibodies**

Fluorophore overview

Molecular Probes fluorescence technologies and cell analysis capabilities have been developed since 1975 and include, from the Alexa Fluor dyes and Qdot labels to Zenon labeling technology. We are able to offer a complete portfolio of Molecular Probes fluorescent labels that spans the near-UV, visible, and near-IR spectrum. This expertise extends to our primary and secondary antibody conjugation capabilities, resulting in brighter, more stable fluorescent conjugates. Regardless of your instrument, we have labels designed to help you get the most out of every sample and every flow cytometry run. See Table 3 for a list of available fluorophores and their specifications.

Classic fluorescent dyes and tandem dyes

A wide variety of fluorescent dye–conjugated primary antibodies is available, including antibodies conjugated with Invitrogen™ Alexa Fluor™ dye, Invitrogen™ Pacific Blue™ dye, Invitrogen™ Pacific Green™ dye, Invitrogen™ Pacific Orange™ dye, fluorescein, fluorescent proteins (PE, PerCP, and APC), and 11 different tandem dye combinations (Table 3). This broad range of dye options becomes even more critical as advances in instrumentation technology allow more and more fluorescent colors to be used (Figure 14 and Table 4).

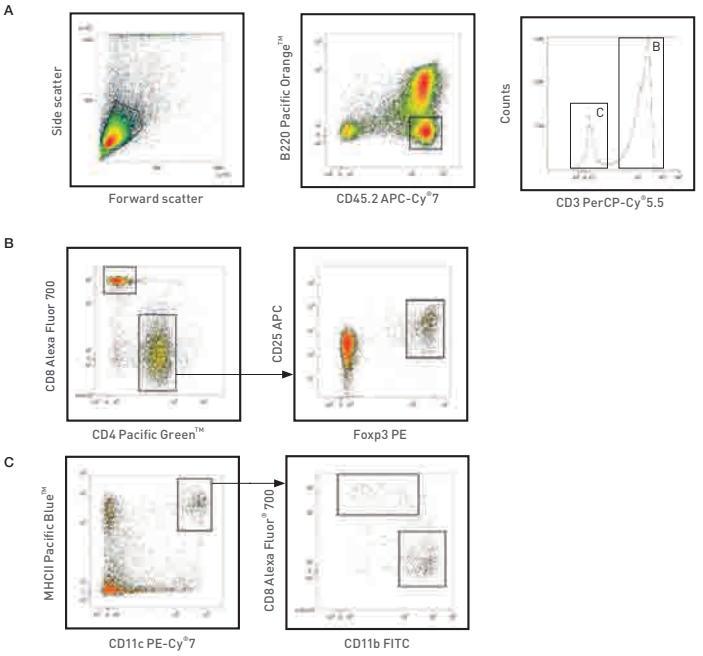


Figure 14. Multiparameter (10-color) analysis of murine regulatory T cells and dendritic cells with the Attune NxT flow cytometer. C57BL/6 splenocytes were surface stained with primary antibodies to the surface antigens listed in Table 4. This step was followed by fixation and permeabilization of the sample using the FIX & PERM Cell Permeabilization Kit (Cat. No. GAS003, GAS004). Intracellular staining was performed during the permeabilization step using PE-conjugated rat anti-mouse Foxp3 antibody. Lymphocytes were gated using FSC/SSC parameters (**A, left**) and B220-expressing B cells were omitted from subsequent analysis (**A, middle**). Within the B220-, CD45.2+ gate, T cells were analyzed based on their expression of CD3 (**A, right**). CD3+ T cells were separated into two populations based on expression of the co-receptors CD4 or CD8 (**B, left**). Within the CD4+ T cells there is a subpopulation of suppressive regulatory T cells that express the transcription factor Foxp3 and the cell surface marker CD25 (IL-2Rα) (**B, right**). CD3- cells were separated to show a rare population of CD11c+ MHCII+, professional antigen-presenting dendritic cells (**C, left**). Splenic dendritic cells can be subdivided further into CD11b+ and CD8+ dendritic cell subsets (**C, right**), each possessing unique antigen presentation properties.

Table 3. Fluorophores for flow cytometry.

Fluorophore	Laser						Emission (nm)
	UV	Violet 405 nm	Blue 488 nm	Green 532 nm	Yellow 561 nm	Red 633/635 nm	
Antibody conjugates							
Alexa Fluor 350	●						442
Alexa Fluor 405	●	●					421
Pacific Blue		●					455
Pacific Green		●					500
Pacific Orange		●					551
Alexa Fluor 488			●				519
Fluorescein (FITC)			●				525
Qdot 605	●	●	●	●	●		605
Qdot 655	●	●	●	●	●		655
Qdot 705	●	●	●	●	●	●	705
Qdot 800	●	●	●	●	●	●	800
Peridinin chlorophyll (PerCP)			●				678
PerCP-Cy5.5			●				695
R-phycoerythrin (R-PE, PE)			●	●	●		575
PE-Texas Red			●	●	●		615
PE-Alexa Fluor 610			●	●	●		628
TRI-COLOR (TC, PE-Cy5)			●	●	●		670
PE-Cy5.5			●	●	●		694
PE-Alexa Fluor 700			●	●	●		723
PE-Cy7			●	●	●		767
Allophycocyanin (APC)						●	660
APC-Cy5.5						●	694
APC-Cy7						●	767
APC-Alexa Fluor 750						●	775
Alexa Fluor 647						●	668
Alexa Fluor 700						●	719
APC-Alexa Fluor 750						●	775

Table 4. Products used in multiparametric analysis of T cells and dendritic cells (Figure 14).

Target*	Host	Fluorophore	Laser	Emission max (nm)	Cat. No.
MHCII	Rat	Pacific Blue dye	Violet	455	A14901
CD4	Rat	Pacific Green dye	Violet	500	C11207
CD45R	Rat	Pacific Orange dye	Violet	551	RM2630
CD11b	Rat	FITC	Blue	525	RM2801
Foxp3	Rat	R-PE	Blue	575	N/A
CD3	Hamster	PerCP-Cy5.5	Blue	695	A14784
CD11c	Hamster	PE-Cy7	Blue	767	A15849
CD25 (IL-2Rα)	Rat	APC	Red	660	NA
CD45.2	Mouse	APC-Cy7	Red	767	A18642
CD8	Rat	Alexa Fluor 700	Red	719	MCD0829

*All products are For Research Use Only. Not for use in diagnostic procedures.
NA-not available for purchase

Qdot antibody conjugates

- Excited by 405 nm or 488 nm, maximizing violet laser use
- Combine with existing organic dyes, increasing the number of detectable parameters
- Do not degrade over time like tandem conjugates, allowing greater reproducibility
- Narrow emission spectra allow for minimal compensation when using a single excitation source

Qdot antibody conjugates possess a bright fluorescence emission that makes them well suited for the detection of low-abundance extracellular proteins. Approximately the same size as R-PE and compatible with existing organic fluorophore conjugates, Qdot antibody conjugates can be excited with any wavelength below their emission maximum, but are best excited by UV or violet light. The narrow, symmetric emission profiles of Qdot antibody conjugates allow for minimal compensation when using a single excitation source, and their very long Stokes shifts enable better, more efficient multicolor assays using the 405 nm violet laser. Available in multiple colors for use in flow cytometry (Figure 15 and Table 5), these advantages make Qdot antibody conjugates powerful tools for antibody labeling and staining.

Learn more about Qdot nanocrystals and their applications in flow cytometry at **thermofisher.com/flow-qdot**

Table 5. Qdot primary antibody conjugates selection guide.

Target*	Clone	Conjugate‡			
		Qdot 605	Qdot 655	Qdot 705	Qdot 800
CD2	S5.5	Q10172	Q22140§	Q22141§	Q22142§
CD3e	S4.1 (AKA 7D6)		Q10012 Q10484§		Q22149§
CD3e	S3.5	Q10054			
CD4	RM4-5	Q10008 Q10480§	Q10007 Q10482§	_Q10060 Q10485§	Q22153§
CD4†	3B5	Q10092	Q22163§	Q22164§	Q22165§
CD8	MEM-78	Q10009 Q10481§	Q10055	Q10059 Q10483§	Q22157§
CD10	TüK4	Q10481§"		Q22136§	
CD14	SJ25-C1	Q10053			Q10064
CD14	6D5	Q10013	Q10056	Q22137§	Q22139§
CD19	CLB-27/1	Q10306	Q10179	Q22138§	Q22162§
CD19†	HIT2	Q22160§	Q10379	Q22161§	
CD27	H130	Q10065	Q10066		Q22152§
CD38	RA3-6B2		Q22150§		Q10156
CD45	MEM-56	Q10051	Q22154§	Q10062	Q22168§
CD45R†	Tü36	Q22166§	Q10176	Q22167§	Q22156§
CD45RA		Q10047	Q10069	Q22155§	Q10063
HLADR (class II)		Q10052	Q22158§	Q22159§	
Isotype control mouse IgG2a		Q10014	Q10015	Q10076	

*Host for all antibodies is mouse unless otherwise indicated. All products are For Research Use Only. Not for use in diagnostic procedures.
†Host is rat.
‡All product sizes are 100 tests unless otherwise indicated.
§Product size is 25 tests.

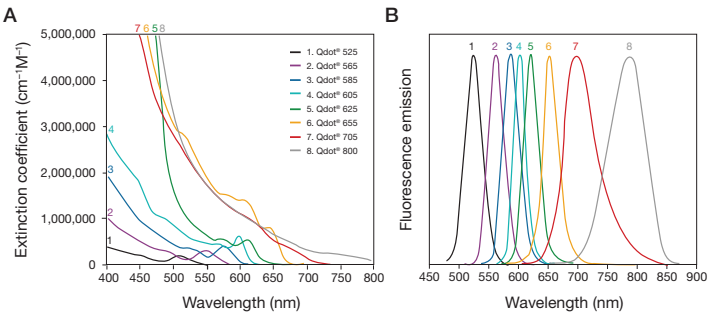


Figure 15. Absorption and emission profiles of Qdot labels. (**A**) Absorption spectra of Qdot labels plotted in terms of molar extinction coefficient. (**B**) Normalized emission spectra of Qdot labels. Numbers are the same as those in panel A.

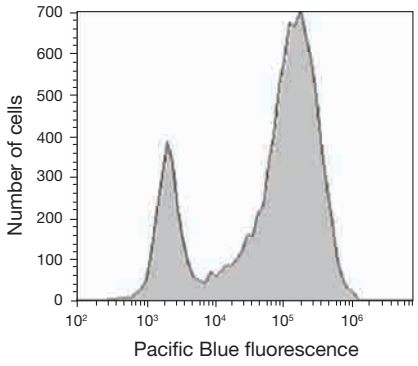


Figure 16. Histogram of gated U2OS cells expressing mCherry protein and labeled with Pacific Blue dye-conjugated rat anti-mCherry antibody (Cat. No. M11238). Samples were acquired and analyzed using 405 nm excitation and 522/31 nm band pass emission filters on an Attune flow cytometer.

mCherry rat monoclonal antibody conjugates

Fluorescent conjugates of our affinity purified rat monoclonal antibodies for mCherry fluorescent protein (Table 6) can be used to detect native and denatured forms of mCherry or mCherry fusion proteins in flow cytometry applications (Figure 16). Full-length mCherry was used as the immunogen, and the resulting monoclonal IgG2a-isotype antibody detects denatured and native forms of the protein. Because of its improved brightness, superior photostability, and extremely rapid maturation rate, mCherry monomeric red fluorescent protein is becoming the red fluorescent protein of choice for monitoring physiological processes and detecting transgene expression.

Table 6. mCherry rat monoclonal antibody (clone 16D7) conjugates.

Dye conjugate	Excitation laser color	Excitation/ Emission (nm)	Cat. No.
Pacific Blue	Violet	405/455	M11238
Alexa Fluor 488	Blue	488/519	M11239
Alexa Fluor 594	Red	590/617	M11240
Alexa Fluor 647	Red	650/668	M11241

Antibody labeling

We offer a number of Molecular Probes labeling kits for the direct attachment of intensely fluorescent Alexa Fluor dyes, Qdot nanocrystals, R-phycoerythrin (R-PE), or even biotin to IgG antibody at levels less than 10 µg up to 1 mg. Directly labeled antibodies allow you to use more than one same-species antibody in a single staining experiment. You can use traditional labeling chemistries optimized for your application or site-specific labeling using click-chemistry technology. Below we describe two options for easy labeling of flow cytometry primary antibodies. To find more information and products, go to **thermofisher.com/flow-antibodylabeling**

The SiteClick antibody labeling system

- Contains reagents to label 100 µg of IgG antibody
- Easy-to-follow step-by-step protocol
- Highly efficient site-specific, reproducible labeling chemistry
- R-PE and Qdot labels for flow cytometry

The Invitrogen™ SiteClick™ system represents a new paradigm for the universal site-selective labeling of antibodies. This modular, click chemistry–mediated method allows you to enzymatically label essentially any antibody on its heavy chain N-linked glycans. In contrast to standard antibody labeling techniques, which can be tedious and inconsistent, the SiteClick site-selective approach produces highly robust and reproducible labeling of antibodies with an impressive choice of detection molecules.

The site-selective labeling provided by the SiteClick system (Figure 17) prevents disruption of the antigen-binding domain that can occur with traditional amine or thiol reactive labeling reagents and eliminates the need to genetically engineer labeling sites into the antibody prior to modification. This site-selective strategy is especially important when labeling monoclonal antibodies that contain lysine residues in or around the antigen-binding domain, as labeling of these sites can disrupt antigen binding. Monoclonal antibodies can also have variable sensitivity to disulfide bond–reducing agents used in reductive cysteine labeling, leaving partially dissociated antibody fragments that result in decreased antibody binding and yield.

We offer antibody labeling kits with R-PE and an array of Qdot labels (Table 7), which are designed especially for flow cytometry applications. The kits are configured to provide an easy-to-follow workflow designed to allow novice and experienced scientists alike to obtain efficient antibody labeling every time (Figure 18). And, because antibody glycans are highly conserved, even between different species, the reproducibility of SiteClick labeling for different antibodies is very high, precluding the need to optimize labeling of each newly acquired antibody. To find out more, go to **thermofisher.com/flow-siteclick**

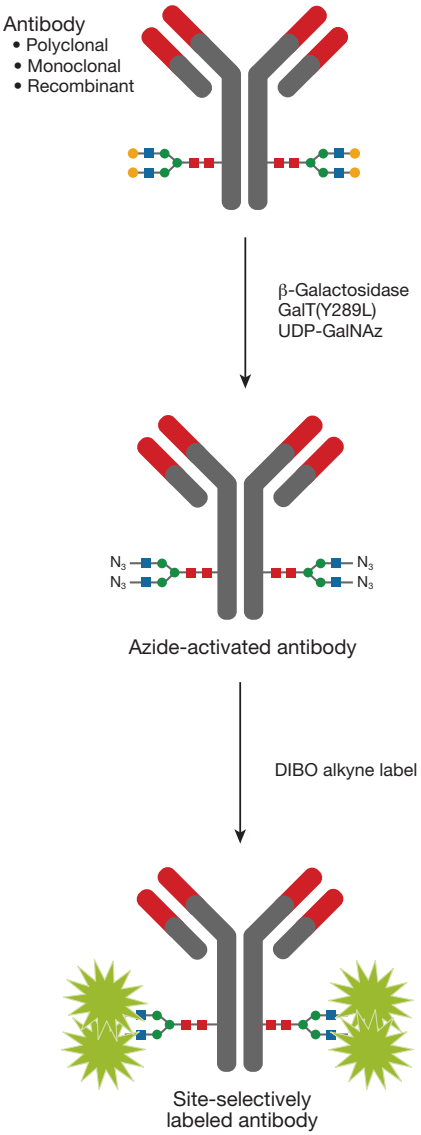


Figure 17. The SiteClick antibody labeling system. The first step in the SiteClick antibody labeling process involves removal of terminal galactose residues from heavy chain N-linked glycans using β-galactosidase, exposing essentially all possible modifiable GlcNAc residues. Second, the free terminal GlcNAc residues are activated with azide tags by enzymatic attachment of GalNAz to the terminal GlcNAc residues using the GalT(Y289L) enzyme. In the third step, the azide residues are reacted with the dibenzocyclooctyne (DIBO)-functionalized probe of choice (e.g., Alexa Fluor 488 DIBO alkyne). The average degree of labeling is 3–3.5 labels per antibody.

Zenon labeling technology

Invitrogen™ Zenon™ labeling technology provides a versatile, easy-to-use system for labeling mouse IgG1, IgG2a, and IgG2b isotypes, as well as rabbit, goat, and human IgG antibodies, with our premier Molecular Probes dyes as well as other fluorophores, biotin, photoproteins, and enzymes. This technology offers several advantages over direct chemical labeling, including:

- Speed—the entire labeling procedure typically takes only 10 minutes
- Efficiency—designed to label nearly 100% of the primary antibodies in solution
- Economy—submicrogram amounts of antibody can be labeled
- Simplicity—no pre- or postlabeling purification of the antibody is required
- Flexibility—easily use multiple primary antibodies in a single experiment

Zenon labeling technology uses a fluorophore-, biotin-, or enzyme-labeled Fab fragment directed against the Fc portion of an intact IgG antibody to form a noncovalent labeling complex. To help ensure their high affinity and selectivity for the Fc portion of the target antibody, the Zenon labeling reagents have been affinity purified during their preparation. Because the Zenon labeling method is based on immunoselectivity, there is no need to remove exogenous proteins or amine containing buffers from the antibody sample prior to forming the complex. Antibodies labeled using Zenon technology display fluorescence intensity or enzymatic activity similar to that observed for directly labeled antibody conjugates.

Our wide selection of Zenon labeling reagents (Table 8) can be mixed and matched, providing the freedom to experiment with multiple dye–antibody combinations in flow cytometry applications. Learn more about the Zenon antibody labeling technology and other antibody labeling products at **thermofisher.com/flow-antibodylabeling**

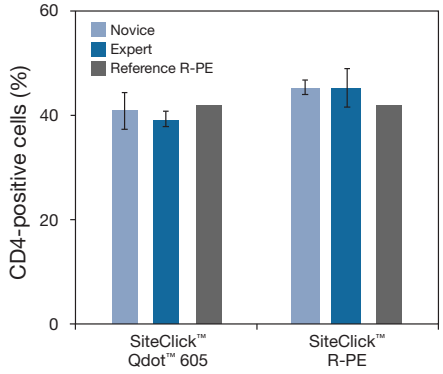


Figure 18. Efficient antibody labeling for both novice and experienced scientists. The SiteClick Qdot 605 Antibody Labeling Kit and SiteClick R-PE Antibody Labeling Kit were used by both a novice user and an expert user to label an anti-CD4 monoclonal antibody in triplicate. The labeled antibodies were used to label CD4-positive cells isolated from a single human blood donor, with subsequent analysis by flow cytometry; the reference sample is a commercially available R-PE anti-CD4 antibody conjugate. The data show percentages of CD4-positive cells relative to total cells, and the error bars indicate variation in the triplicate kit labelings for each user. The novice user had never before performed protein bioconjugation, yet obtained labeling efficiencies equivalent to those obtained by an expert user.

Table 7. SiteClick antibody labeling kits.

Product	Excitation laser color	Emission (nm)	Cat. No.
SiteClick R-PE Antibody Labeling Kit	Blue	578	S10467
SiteClick Qdot 525 Antibody Labeling Kit	UV, violet, blue	525	S10449
SiteClick Qdot 565 Antibody Labeling Kit	UV, violet, blue, green	565	S10450
SiteClick Qdot 585 Antibody Labeling Kit	UV, violet, blue, green, yellow	585	S10451
SiteClick Qdot 605 Antibody Labeling Kit	UV, violet, blue, green, yellow	605	S10469
SiteClick Qdot 625 Antibody Labeling Kit	UV, violet, blue, green, yellow	625	S10452
SiteClick Qdot 655 Antibody Labeling Kit	UV, violet, blue, green, yellow	655	S10453
SiteClick Qdot 705 Antibody Labeling Kit	UV, violet, blue, green, yellow, red	705	S10454
SiteClick Qdot 800 Antibody Labeling Kit	UV, violet, blue, green, yellow, red	800	S10455

Table 8. Zenon labeling kit selection guide.

Dye	Ex/Em*	Mouse IgG1	Mouse IgG2a	Mouse IgG2b	Rabbit IgG	Goat IgG	Human IgG
Alexa Fluor Dyes†							
Alexa Fluor 350	346/442	Z25000	Z25100		Z25300		
Alexa Fluor 405	402/421	Z25013	Z25113	Z25213	Z25313		
Alexa Fluor 488	495/519	Z25002	Z25102	Z25202	Z25302	Z25602	Z25402
Alexa Fluor 546	556/573	Z25004			Z25304		
Alexa Fluor 555	555/565	Z25005	Z25105	Z25205	Z25305	Z25605	
Alexa Fluor 568	578/603	Z25006	Z25106		Z25306		
Alexa Fluor 594	590/617	Z25007	Z25107	Z25207	Z25307	Z25607	Z25407
Alexa Fluor 647	650/668	Z25008	Z25108	Z25208	Z25308	Z25608	Z25408
Alexa Fluor 680	679/702	Z25010	Z25110	Z25210			
Alexa Fluor 700	696/719	Z25011					
Classic Dyes†							
Pacific Blue	410/455	Z25041	Z25156				
Pacific Green	411/500	Z11203					
Pacific Orange	400/551	Z25256	Z25257				
Fluorescein	494/518	Z25042			Z25342		
Biotin†							
Biotin-XX	NA	Z25052	Z25152	Z25252	Z25352		Z25452
Phycobiliproteins and Tandem Dyes‡							
R-PE	496§/578	Z25055	Z25155	Z25255	Z25355		Z25455
R-PE-Alexa Fluor 610	496§/630	Z25020					
R-PE-Alexa Fluor 647	496§/668	Z25021					
Allophycocyanin (APC)	650/660	Z25051	Z25151	Z25251	Z25351		Z25451
APC-Alexa Fluor 750	650/775	Z25031					
Enzymes‡							
Horseradish peroxidase	NA	Z25054	Z25154	Z25254	Z25354		

*Approximate fluorescence excitation and emission maxima, in nm.
†Each Zenon labeling kit with an Alexa Fluor dye, classic dye, or biotin contains materials for 50 labeling reactions; one labeling reaction is defined as the amount of Zenon reagent required to label 1 µg of antibody.
‡Each Zenon labeling kit with a phycobiliprotein or enzyme contains materials for 25 labeling reactions; kits with tandem dyes contain materials for 10 labeling reactions.
§Additional excitation peaks are present at 546 and 565 nm.
NA, not applicable.
Go to **thermofisher.com/flow-zenon** for more information.

Secondary detection

Our extensive selection of secondary detection reagents includes antibodies and streptavidin labeled with our superior Alexa Fluor dyes, phycobiliproteins, Alexa Fluor dye–phycobiliprotein tandem fluorophores (Figure 19), Qdot labels, biotin, and enzyme labels (horseradish peroxidase and alkaline phosphatase). We also offer antibodies with different immunoreactivities, essential to avoid confounding cross-reactivity when performing simultaneous secondary immunodetection of two or more targets.

Find a small selection of conjugates in Table 9. Use our online Secondary Antibody Selection Tool to find the right secondary detection reagent. With this tool, you can specify target IgG class (and form), host, species reactivity, and conjugate type to narrow your results. Find your secondary antibody now at **thermofisher.com/flow-secondarydetection**

Table 9. Secondary detection reagent selection guide.

Dye	Dye or label									
	Alexa Fluor 405	Pacific Blue	Pacific Green	Pacific Orange	Alexa Fluor 488	PE	Alexa Fluor 610–R-PE	Alexa Fluor 647–R-PE	APC	Alexa Fluor 750–APC
Anti-mouse IgG	A31553	P10993	P11204		A11001	P852	A20980	A20990	A865	A21006
Anti-rabbit IgG	A31556	P10994		P31584	A11008	P2771MP	A20981	A20991	A10931	
Anti-rat IgG					A11006	A10545			A10540	
Streptavidin	S32351	S11222	S11200	S32365	S11223	S866	S20982	S20992	S868, S32362*	S21008

*Premium grade.

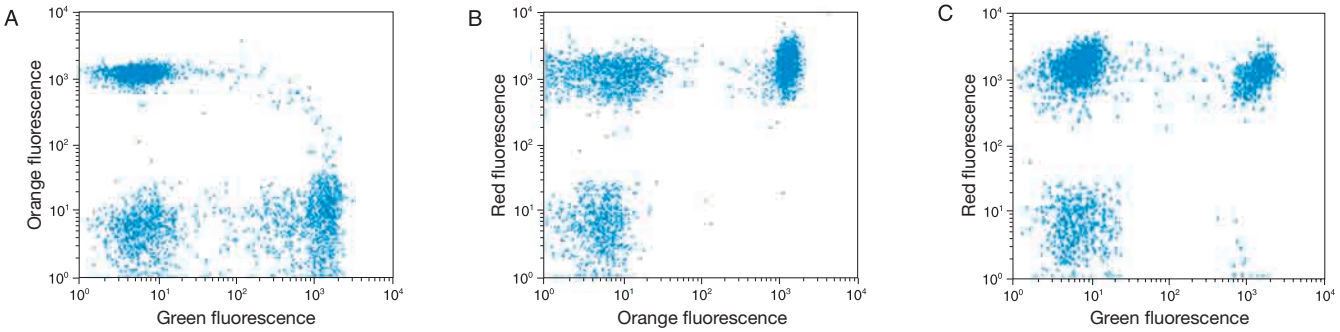


Figure 19. Simultaneous detection of three cell surface markers using an Alexa Fluor 610–R-phycoerythrin tandem streptavidin conjugate, Alexa Fluor 488 dye and R-phycoerythrin labels. Lymphocytes from ammonium chloride red blood cell–lysed whole blood were labeled with a biotinylated mouse anti-human CD3 antibody, washed with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), and then incubated with Alexa Fluor 610–R-phycoerythrin tandem dye–labeled streptavidin (Cat. No. S20982). Cells were again washed and then labeled with Alexa Fluor 488–dye conjugated anti-human CD8 antibody and PE-conjugated anti-human CD4 antibody. After a further wash in 1% BSA/PBS, labeling was analyzed on a Becton Dickinson FACScan flow cytometer using excitation at 488 nm. CD8 was detected in the green channel (525 + 10 nm), CD4 in the orange channel (575 + 10 nm) and CD3 in the red channel (>650 nm). The bivariate scatter plots show the expected mutually exclusive populations of (**A**) CD4 and CD8 positive cells, together with (**B**) co-positive CD3/CD4 and (**C**) CD3/CD8 populations.

Custom services for flow cytometry antibodies

Our custom antibody service is efficient, and we stand behind the quality of our work. Dedicated project managers will guide your project through every step of the process, and constantly keep you informed of our progress. Let us take the hassle out of your hands with a custom solution for you.

We offer custom antibody services that include:

- Conjugation—using your antibody or one of ours, with the largest selection of labels available
- Formulation—including azide-free, different buffers, or different concentrations
- Packaging—bulk quantities
- Mixtures—research antibody cocktails

For more information, please email Custom Services at custom.services@thermofisher.com

Cell analysis

An extensive array of Molecular Probes stains and kits have been developed to assess cell function, health, and viability. Whether the health of cells is your primary question or simply a critical factor in getting the right answers to other questions, we have a solution for you. For more information and more products, go to **thermofisher.com/flow-cellhealth**

Cell viability

Cell viability assays can be used to simply distinguish between live and dead cell populations, to correlate with other cell functions or treatments, or to exclude dead cell populations from analyses. Below we describe two different cell viability assay types that use only one channel of the flow cytometer, making them especially useful with multicolor flow cytometry (Table 10). For more information and more products go to **thermofisher.com/flow-cellviability**

LIVE/DEAD Fixable Dead Cell Stain Kits

- Staining retained after fixation for simple live/dead analysis with intracellular phenotyping
- Fits into almost any staining and phenotyping protocol
- Seven colors to choose from for UV, 405, 488, or 633 lasers

The LIVE/DEAD Fixable Dead Cell Stain Kits (see the product list, page 30) covalently bind available amino acids but are excluded from the cytosol of live, healthy cells. The dyes react with surface proteins of both live and dead cells, but label proteins throughout the cytoplasm of cells with compromised membranes, causing dead cells to fluoresce at least 50 times brighter than live cells. Because the labeling is covalent, stained cells can be fixed and permeabilized without losing the viability discrimination signal (Figure 20), making these reagents ideal if you want to fix and permeabilize samples and maintain dead-cell discrimination during subsequent analysis. LIVE/DEAD Fixable Dead Cell Stains are available to match a range of excitation sources and detection channels. For compensation control, use the LIVE/DEAD Fixable Dead Cell Stains with the ArC Amine Reactive Compensation Bead Kit (Cat. No. A10346; see page 8) for optimal results.

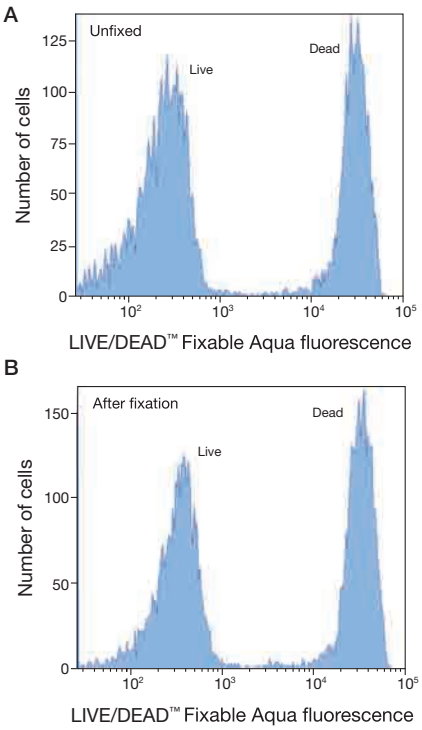


Figure 20. Retention of LIVE/DEAD Fixable Dead Cell Stains after fixation. The LIVE/ DEAD Fixable Aqua Dead Cell Stain Kit (Cat. No. L34957) was used to differentially stain a mixture of live (left peak) and heat-treated Jurkat cells (right peak). Cells in (**A**) were not fixed; cells in (**B**) were fixed in 3.7% formaldehyde following staining. Samples were analyzed by flow cytometry using 405 nm excitation and ~525 nm emission.

Table 10. Cell viability assay selection guide.

Product	Target	Fixable?*	No-wash?	Live cell fluorescence	Dead cell fluorescence	Applications
LIVE/DEAD Fixable Dead Cell Stains	Surface and intracellular proteins	Yes	No	Very dim	Very bright	Immunophenotyping
SYTOX Dead Cell Stains	Nucleic acids	No	Yes	Very dim	Very bright	Live cell analyses, dead cell exclusion
Propidium Iodide Ready Probes Reagent	Nucleic acids	No	Yes	Very dim	Very bright	Live cell analyses, dead cell exclusion

*Formaldehyde fixation only.

The inadvertent inclusion of sick or dead cells in experiments can dramatically affect the outcomes. For instance, including dead cells in immunophenotyping analysis can distort the results, especially for rare phenotypes. Perfetto et al. (2006) showed that light-scatter gating during flow cytometry is not enough to exclude all dead cells from analysis during leukocyte immunophenotyping (see Figure 21 for citation). Using LIVE/DEAD Fixable Dead Cell Stains, they were able to efficiently exclude dead cells from analysis and, consequently, significantly increase accuracy in their assays.

SYTOX Dead Cell Stains

- High-affinity nucleic acid stains for easy dead-cell discrimination
- Multiple colors with minimal spectral overlap for expanded multicolor capabilities
- No wash steps; just add, incubate, and analyze, for simplified protocols

Invitrogen™ SYTOX™ Dead Cell Stains (see product list, page 30) are excluded from cells with intact membranes but quickly diffuse into cells that have compromised membranes. Once inside, these dyes bind DNA, which produces a significant enhancement of their fluorescence; live cells remain nonfluorescent and dead cells fluoresce brightly. SYTOX stains are available to match widely available excitation sources. These dyes are often used in a "dump channel," with gating on the viable cells for further analysis (Figure 22). No wash step is required; in fact, SYTOX Dead Cell Stains do not bind covalently to DNA, so dye concentrations must be maintained during analysis.

Propidium Iodide ReadyProbes Reagent

Propidium iodide (PI) is a commonly used dead-cell stain that emits red fluorescence when bound to DNA. With Invitrogen™ Propidium Iodide ReadyProbes™ Reagent (Cat. No. R37108), we provide a room temperature–stable solution of the classic DNA stain that comes in a convenient-to-use dropper bottle. Just tip and drip two drops per ml to stain your cells.

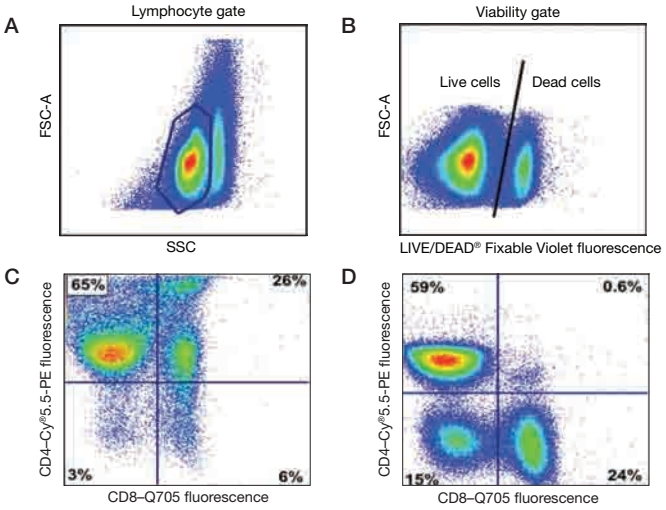


Figure 21. Exclusion of dead cells eliminates staining artifacts from analysis. After the application of a lymphocyte gate (**A**), live and dead cells were discriminated (**B**) using LIVE/DEAD Fixable Violet Dead Cell Stain Kit (Cat. No. L34955). Note the significant number of dead cells despite a scatter gate. Subsequent analysis of dead cells (**C**) and live cells (**D**) shows the dramatic difference in apparent phenotypes between the two cell populations. Reprinted from Perfetto SP, Chattopadhyay PK, Lamoreaux L, et al. (2006) *J Immunol Methods* 313:199–208, with permission from Elsevier.

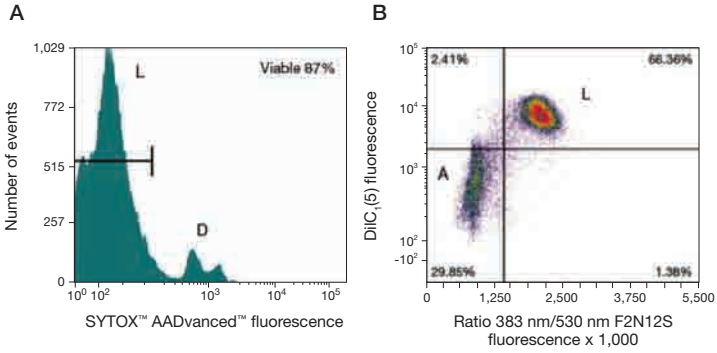


Figure 22. Cell viability detected using SYTOX AADvanced Dead Cell Stain. Jurkat cells were treated with camptothecin for 4 hours then stained with 1 µM SYTOX AADvanced Dead Cell Stain (Cat. No. S10349), 200 nM F2N12S (Cat. No. A35137), and 50 nM MitoProbe™ DiIC1(5) (Cat. No. M34151). (**A**) Dead cells were first excluded by gating live cells (those that have lower SYTOX AADvanced fluorescence, indicated by bars). (**B**) Bivariant density plot shows a two-parameter apoptosis assay for mitochondrial membrane potential loss (decreased DiIC1(5) fluorescence) and breakdown of membrane asymmetry (smaller F2N12S 585/530 nm fluorescence ratio). The **A, L, and D** labels on the graphs indicate apoptotic, live, and dead cells, respectively. Control data are not shown.

Cell proliferation

Cell proliferation and the characterization of agents that either promote or retard cell proliferation are extremely important areas of cell biology and drug discovery research. We offer both traditional reagents for assessing cell proliferation (CellTrace Cell Proliferation Kits), as well as the latest technology for measuring new DNA synthesis (Click-iT Plus EdU labeling) (Table 11). For more information and more products, go to **thermofisher.com/flow-cellproliferation**

Click-iT Plus EdU Flow Cytometry Assay Kits

- Superior accuracy compared to BrdU assays, with minimal variation (low CV values)
- Streamlined five-step protocol
- Multiplexable with GFP, mCherry, APC, PerCP, PE, and other fluorophores

The growth of cells within a population can be indirectly observed by measuring modified nucleoside incorporation into newly synthesized DNA. EdU (5-ethynyl-2´-deoxyuridine) is a nucleoside analog that is incorporated into DNA during synthesis. The Invitrogen™ Click-iT™ Plus EdU Flow Cytometry Assay Kits allow detection of EdU (which contains an alkyne) by a copper-catalyzed reaction that produces a stable covalent bond between the alkyne and a fluorescent dye–labeled picolyl azide. The small size of the picolyl azide detection reagents means that the fluorescent label has efficient access to the intact DNA without the need for harsh cell treatment.

In the past, DNA synthesis was measured by incorporating the nucleoside analog bromodeoxyuridine (BrdU) into DNA, followed by detection with an anti-BrdU antibody. Although useful in its time, that method requires DNA denaturation (using acid, heat, or DNase) to expose the BrdU to the antibody—a step that can adversely affect sample quality. The Click-iT Plus EdU Flow Cytometry Assay eliminates the need to denature DNA, thus simplifying the assay considerably yet generating comparable results (Figure 23). Click-iT Plus EdU labeling is compatible with fixation protocols.

The Click-iT Plus kits can be used with fluorescent proteins, because the Click-iT Plus reaction uses a modified azide in place of the azide used in the original Click-iT reaction. As a result of the modification, the concentration of free copper in the sample is significantly lower and fluorescence signals from fluorescent proteins (e.g., R-PE, R-PE tandem dyes, and GFP) are not quenched. The speed and accuracy of the Click-iT Plus EdU reaction is comparable to that of the original Click-iT EdU reaction. See the product list on page 30 for available kits.

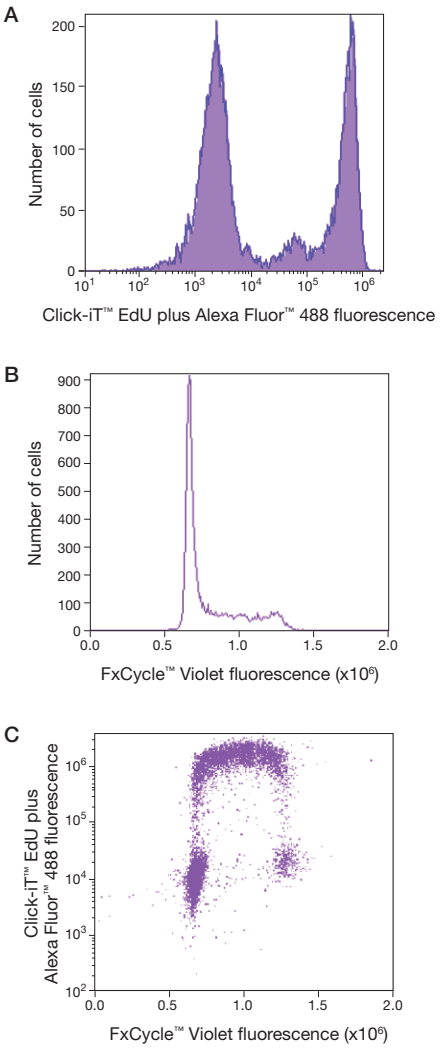


Figure 23. Cell proliferation analysis using the Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay Kit and FxCycle Violet Stain. Jurkat cells were treated with 10 µM EdU for one hour and stained with Alexa Fluor 488 picolyl azide, according to the Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay Kit's protocol (Cat. No. C10632), followed by staining with FxCycle Violet Stain (Cat. No. F10347). Cells were then analyzed by flow cytometry using either 488 nm excitation (for Click-iT EdU Alexa Fluor 488 dye) or 405 nm excitation (for FxCycle Violet Stain). (**A**) Histogram demonstrating clear separation of cells in S phase (DNA synthesis, including EdU incorporation) and cells in either G2/M or G0/G1. (**B**) Histogram showing DNA content distribution, with G0/G1 and G2/M phase peaks separated by the S phase distribution using FxCycle Violet Stain. (**C**) Dual-parameter Click-iT Plus EdU and FxCycle plot shows co-positive cells that provide a direct measurement of the percentage of cells in S phase.

Table 11. Cell proliferation assay selection guide.

Product	Target	Fixable	Multiplexing	Application
Click-iT Plus EdU Flow Cytometry Assay Kits	Incorporation into newly synthesized DNA	Yes	Yes	Cell proliferation
CellTrace Cell Proliferation Kits	Lysine-containing proteins	Yes	Yes	Generational analysis

CellTrace Cell Proliferation Kits

- Bright single-peak staining enables visualization of multiple generations
- Well retained in cells for several days post-staining
- No known cytotoxic effect on proliferative ability or biology of cells
- Multiple colors available to easily combine with antibodies or markers of cell function, such as GFP
- Simple, robust staining protocol

The Invitrogen™ CellTrace™ family of dyes comprises CellTrace CFSE dye (Cat. No. C34554), CellTrace Violet dye (Cat. No. C34557), and CellTrace Far Red dye (Cat. No. C34564), all of which spontaneously and irreversibly couple to cellular proteins by reaction with lysine side chains and other available amines. When cells divide, the CellTrace dye labeling is distributed equally between the daughter cells, and each successive generation in a population of proliferating cells is marked by a halving of cellular fluorescence intensity. Eight to ten successive generations have been identified with both CellTrace CFSE and CellTrace Violet dye (Figure 24). All of the CellTrace dyes can be used in combination with other cell function probes or markers. Because CellTrace Violet and CellTrace Far Red use lasers and detection channels different from those used for green fluorescence, multiplexing with GFP, fluorescein, or other green-fluorescent probes is possible.

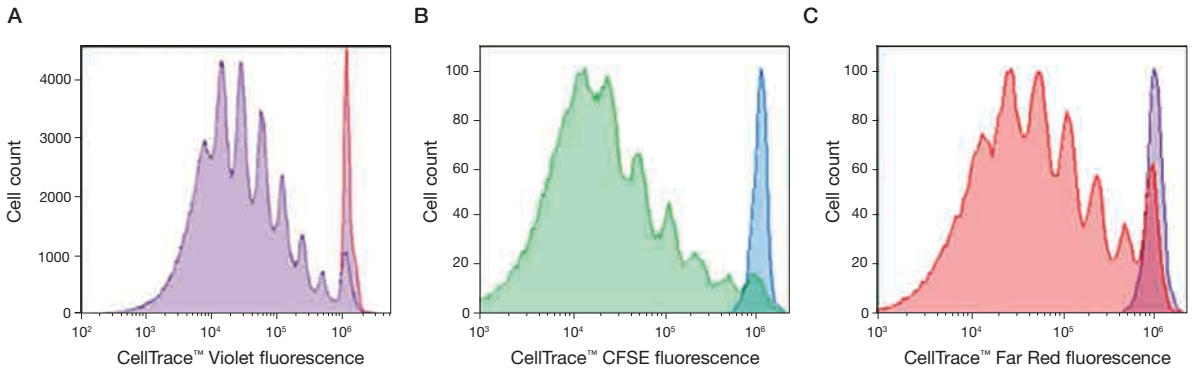


Figure 24. Generational tracing using CellTrace reagents. Cell proliferation was followed for varying numbers of generations under different experimental conditions using (**A**) CellTrace Violet reagent in human peripheral blood mononuclear cells (PBMCs) stimulated for 7 days; (**B**) CellTrace CFSE reagent in human T lymphocytes stimulated for 5 days; and (**C**) CellTrace Far Red reagent in human T lymphocytes stimulated for 5 days. PBMCs were stimulated with anti-human CD3 antibody (Cat. No. MHCD0300) and interleukin-2 (Cat. No. PHC0027) while human T lymphocytes were stimulated with anti-human CD3 antibody (Cat. No. MHCD0300) alone. Unstimulated parent generations are represented by (**A**) red peak; (**B**) blue peak and (**C**) purple peak. Dead cells were excluded from each data group using SYTOX Dead Cell Stains. Analyses were performed using an Attune flow cytometer with the following excitation: bandpass emission filter for each reagent: 405 nm: 450/40 nm for CellTrace Violet detection; 488 nm: 530/30 nm for CellTrace CFSE detection; and 638 nm: 660/20 nm for CellTrace Far Red detection.

Cell cycle

Detection of DNA content provides a snapshot of cells in a population that are in different stages of the cell cycle. Flow cytometry, in conjunction with modeling algorithms, provides a powerful tool to assess cells in G0/G1 phase versus S phase, G2/M phase, or showing polyploidy. Molecular Probes fluorescent dyes allow accurate cell cycle analysis in either live or fixed cell populations (Table 12). For more information and to see more products, go to **thermofisher.com/flow-cellcycle**

Vybrant DyeCycle Stains

- Enables accurate cell cycle analysis in living cells
- Low cytotoxicity for cell sorting and additional live cell experiments
- Multiple color options for easier multiplexing
- Ability to sort based on phase of cell cycle
- Ability to identify stem cell side populations using the violet laser

Invitrogen™ Vybrant™ DyeCycle™ stains offer the ability to stain for DNA profiling in live cells, with options for 405, 488, 532, or 633 nm excitation. The dyes are generally used in combination with a dead-cell stain (Figure 25) to exclude dead cells from the analysis, but the Vybrant DyeCycle stains are not cytotoxic, allowing stained cells to be sorted and then cultured or assessed with functional assays after determining their cell cycle stage. See the product list on page 30 for the available reagents.

FxCycle Stains

- Allows increased flexibility for multicolor cell cycle studies
- Requires little or no compensation with 488 nm excitable dyes
- Tight CV values enable more accurate analysis

Cell cycle analysis with Invitrogen™ FxCycle™ Violet Stain (Cat. No. F10347), FxCycle PI/RNase Staining Solution (Cat. No. F10797), or FxCycle Far Red Stain (Cat. No. F10348) allows easy multiplexing with other cellular assays, such as those measuring cell proliferation (Figure 23, page 22), immunophenotype, apoptosis, or viability, by freeing up other lasers and detection channels. The FxCycle stains, intended for cells that are fixed and permeabilized, provide fluorescence signals proportional to the DNA content of each cell in a population.

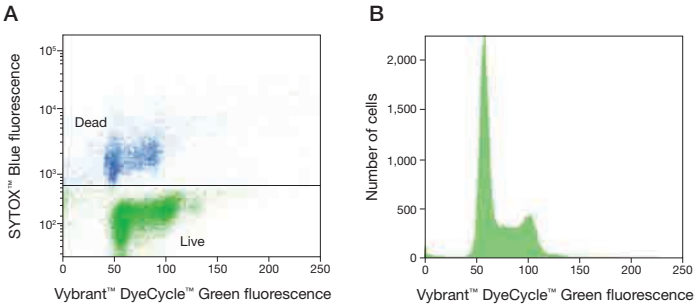


Figure 25. Viable-cell gating with Vybrant DyeCycle Stains. Jurkat cells from an overgrown culture were stained with Vybrant DyeCycle Green Stain (Cat. No. V35004) and then SYTOX Blue Dead Cell Stain (Cat. No. S34857) and analyzed by flow cytometry using 488 nm and 405 nm excitation. The histogram (**B**) was gated on live cells (**A**) and shows DNA content distribution in live cells: G0/G1 and G2/M phase peaks are separated by the S phase distribution. Inclusion of the dead cells would have produced aberrant results.

Table 12. Cell cycle assay selection guide.

Product Name	Target	Live cells?	Multiplexing?	Application
Vybrant DyeCycle Stains	DNA	Yes	Yes	Live cell analysis
FxCycle Stains	DNA	No	Yes	Fixed cell analysis

Apoptosis

Apoptosis is distinct from necrosis in both the biochemical and the morphological changes that occur. In contrast to necrotic cells, apoptotic cells are characterized morphologically by compaction of the nuclear chromatin, shrinkage of the cytoplasm, and production of membrane-bound apoptotic bodies. Biochemically, apoptosis is distinguished by fragmentation of the genome and cleavage or degradation of several cellular proteins. As with cell viability, no single parameter fully defines cell death in all systems; therefore, it is often advantageous to use several different approaches when studying apoptosis. Below we describe several methods and Molecular Probes products for assessing apoptosis (Table 13). For more information and more products for apoptosis, go to **thermofisher.com/flow-apoptosis**

CellEvent Caspase-3/7 Green Flow Cytometry Assay Kit

- Optimized caspase-3/7 substrate for apoptosis analysis
- Simple, no-wash protocol helps preserve delicate apoptotic cells
- Compatible with both live-cell fluorescence imaging and formaldehyde-based fixation methods

Caspases are a family of enzymes that play key roles in initiating and effecting apoptosis, and activation of caspase enzymes is a distinctive feature of the early stages of apoptosis. We provide a variety of caspase assays for flow cytometry, including the Invitrogen™ CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit (Cat. No. C10427), which detects caspase activity with a substrate that, after being cleaved by caspase-3 or caspase-7, binds to DNA and becomes brightly fluorescent (Figure 26).

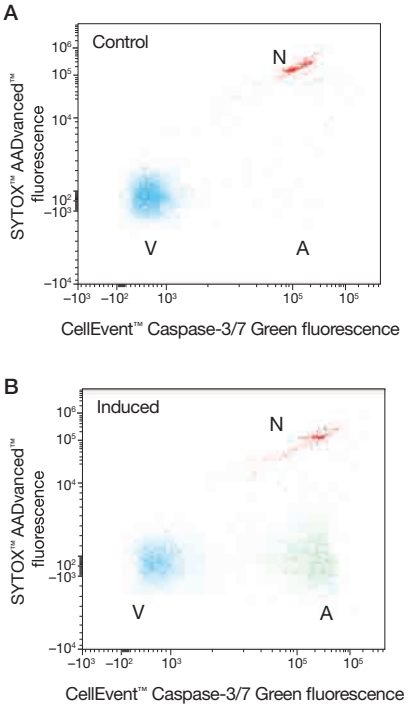


Figure 26. Detection of caspase activity in Jurkat cells, using the CellEvent Caspase-3/7 Green Flow Cytometry Assay Kit. Jurkat cells (human T cell leukemia) were treated with (**A**) DMSO or (**B**) 10 µM camptothecin for 3 hours before labeling with the CellEvent Caspase-3/7 Green Flow Cytometry Assay Kit (Cat. No. C10427). Stained samples were analyzed on the Attune flow cytometer equipped with a 488 nm laser, and fluorescence emission was collected using a 530/30 nm bandpass filter for the CellEvent reagent and a 690/50 nm bandpass filter for the SYTOX AADvanced stain (also provided in the kit). Note that treated cells have a higher percentage of apoptotic cells (**B**) than the basal level of apoptosis seen in the control cells (**A**). **A** = apoptotic cells; **N** = necrotic cells; **V** = viable cells.

Table 13. Apoptosis assay selection guide.

Product name	Target	Fixable	Multiplexing	No. of channels used
CellEvent Caspase-3/7 Green Flow Cytometry Assay Kit	Caspase 3/7	No	Yes	1
Annexin V conjugates	Phosphatidylserine translocation	Yes	Yes	1
MitoProbe JC-1 Assay Kit	Mitochondrial membrane potential	No	Yes	2
Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit	Asymmetric membranes	No	Yes	3

Annexin V conjugates and kits

- Conjugated to Molecular Probes dyes for increased sensitivity
- Conjugates for all available lasers for increased multiplexing capabilities
- Available as stand-alone reagents or easy-to-use kits

Another hallmark of apoptosis is the translocation of phosphatidylserine (PS) from the cytoplasmic surface of cell membranes, where it normally resides, to the external surface of cells. Once this translocation has occurred in apoptotic cells, the PS can be detected by the binding of fluorescently-labeled annexin V, a PS-binding protein. We offer an array of fluorescent conjugates of annexin V, including conjugates of Molecular Probes bright and photostable Alexa Fluor dyes (Figure 27) and an Annexin-binding Buffer (Cat. No. V13246) designed to facilitate PS binding. Additionally, we have flow cytometry–optimized kits that combine some of our other viability and cell function probes with annexin to provide multiparametric data on the apoptotic state of cells. Three of those kits and several annexin V fluorescent conjugates are featured in the product list on page 31. To find all of the available apoptosis kits, go to **thermofisher.com/flow-annexin**

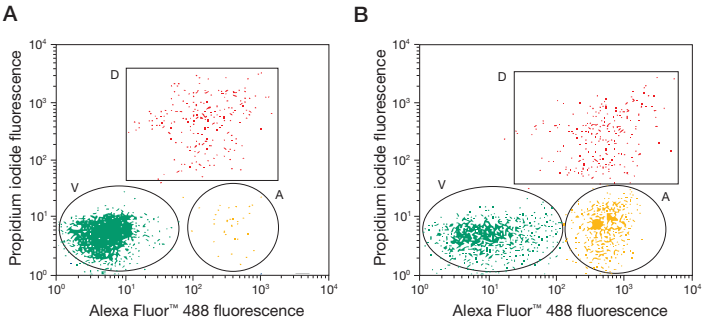


Figure 27. Flow cytometric analysis of Jurkat cells using the Alexa Fluor 488 annexin V conjugate and propidium iodide. Jurkat human T cell leukemia cells were first exposed to 10 µM camptothecin for four hours (**A**) or left untreated (as control, **B**). Cells were then treated with the reagents in the Invitrogen™ Dead Cell Apoptosis Kit with Annexin V Alexa Fluor 488 & Propidium Iodide (Cat. No. V13245) and analyzed by flow cytometry. Note that the camptothecin-treated cells have a significantly higher percentage of apoptotic cells (labeled "**A**") than the basal level of apoptosis seen in the control cells. **V** = viable cells; **D** = dead cells.

MitoProbe JC-1 Assay Kit

- Easy to use and compatible with existing research protocols
- Can be used with multiple cell types
- Available for 488 nm and 633/635 nm excitation
- Low compensation alternatives

The Invitrogen™ MitoProbe™ JC-1 Assay Kit (Cat. No. M34152) provides the cationic dye JC-1, carbonyl cyanide 3-chlorophenylhydrazone (CCCP, a mitochondrial membrane–potential uncoupler), dimethylsulfoxide (DMSO), and concentrated phosphate-buffered saline (PBS) for the study of mitochondrial membrane potential. JC-1 exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~529 nm) to red (~590 nm), due to concentration-dependent formation of red-fluorescent J-aggregates (Figure 28). Consequently, mitochondrial depolarization is indicated by a decrease in the red-to-green fluorescence intensity ratio, which is dependent only on the membrane potential and not on other factors such as mitochondrial size, shape, or density, which may influence single-component fluorescence measurements. Use of fluorescence ratio detection therefore allows researchers to make comparative measurements of membrane potential and to determine the percentage of cells within a population that respond to an applied stimulus.

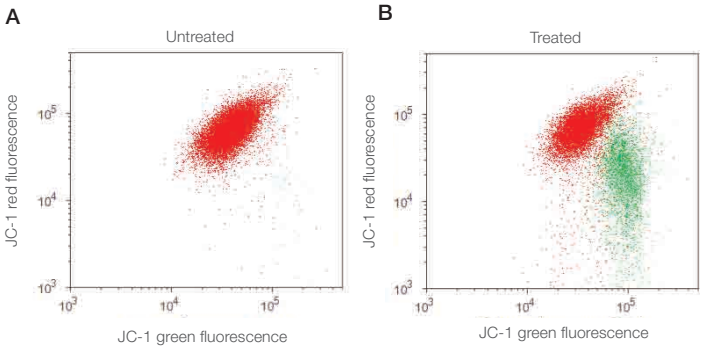


Figure 28. Jurkat cells stained with 2 µM JC-1. Cells were stained using reagents in the MitoProbe JC-1 Assay Kit (Cat. No. M34152) for 20 minutes at 37°C and 5% CO_2, washed with PBS, and analyzed on the Attune flow cytometer using 488 nm excitation with 530/30 nm bandpass and >640 nm longpass emission filters. Untreated cultured cells (**A**) are shown compared to treated cells (**B**), which were induced to undergo apoptosis with 10 µM camptothecin for 5 hours at 37°C.

Membrane asymmetry probe

- Enables accurate apoptotic analysis on trypsinized cells
- Simple 5-minute staining protocol
- Compatible with other blue-excited apoptotic stains

The Invitrogen™ Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit (Cat. No. A35137) provides an easy, efficient method for the detection of apoptosis with dead-cell discrimination using a violet laser flow cytometer (Figure 29). The Violet Ratiometric Membrane Asymmetry Probe detects the membrane asymmetry changes during apoptosis. It works well on adherent and suspension cells, and correlates with other indicators of apoptosis, such as caspase detection and changes in mitochondrial membrane potential. The dye exhibits an excited-state intramolecular proton transfer reaction resulting in dual fluorescence, with two emission bands corresponding to 530 nm and 585 nm, producing a two-color ratiometric response to variations in surface charge. The F2N12S probe is combined with SYTOX AADvanced dead cell stain, which is capable of passing through the cell membrane only in late apoptotic or necrotic cells, allowing discrimination from early apoptotic cells.

Unlike annexin-based assays, this assay does not require special buffers or wash steps, and it is less susceptible to the cell membrane damage commonly found during the physical or chemical removal steps when assaying adherent cells, therefore providing better data quality.

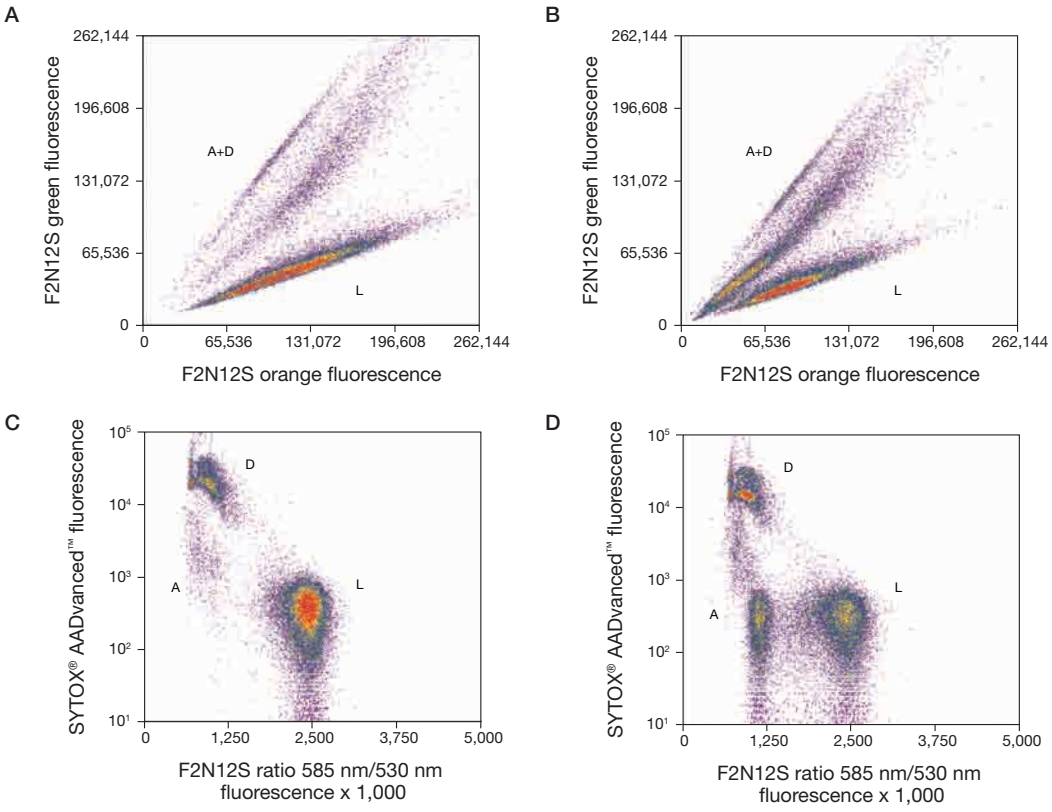


Figure 29. Violet ratiometric membrane asymmetry probe for apoptosis detection. Jurkat cells (T cell leukemia, human) were treated with 10 µM camptothecin for 4 hours (panels **B** and **D**) or left untreated as a control (panels **A** and **C**). Samples were analyzed on a flow cytometer with 405 nm 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. Live cells can be discriminated from apoptotic and dead cells by the relative intensities of the two emission bands from F2N12S (**A** and **B**). 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.

Other cell function assays

CellROX Flow Cytometry Kits

- Fluorogenic probe that is oxidized to a fluorescent form in the presence of reactive oxygen species (ROS)
- Minimal overlap with fluorophores excited by other laser lines, allowing easy multiplexing
- Cells can be stained in complete media or other appropriate buffer; no need for serum-free media

Generation of ROS is inevitable for aerobic organisms and, in healthy cells, occurs at a controlled rate. Under conditions of oxidative stress, ROS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids. Oxidative damage of these biomolecules is associated with aging, as well as with a variety of pathological events, including atherosclerosis, carcinogenesis, ischemia-reperfusion injury, and neurodegenerative disorders.

Invitrogen™ CellROX™ reagents are fluorogenic probes for measuring generalized oxidative stress in cells, using conventional fluorescence microscopy, high-content screening, microplate fluorometry, or flow cytometry (Figure 30). In a reduced state, the dyes are nonfluorescent, but upon oxidation, they fluoresce brightly. Molecular Probes kits for flow cytometry include CellROX Green Flow Cytometry Assay Kit (Cat. No. C10492), CellROX Orange Flow Cytometry Assay Kit (Cat. No. C10493), and CellROX Deep Red Flow Cytometry Assay Kit (Cat. No. C10491). For more information, go to **thermofisher.com/flow-cellrox**

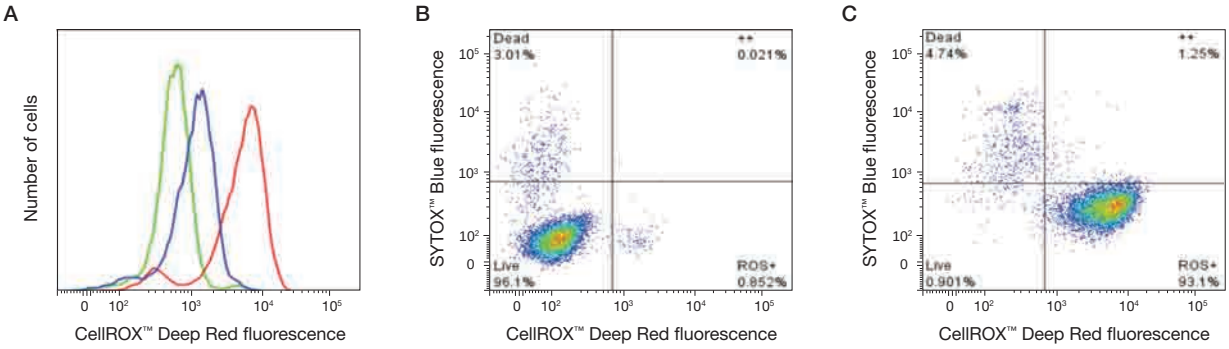


Figure 30. ROS detection by flow cytometry. (**A**) ROS levels detected by the CellROX Deep Red Reagent (provided in the CellROX Deep Red Flow Cytometry Assay Kit, (Cat. No. C10491) are decreased in oxidant-treated Jurkat cells with pretreatment of cultures using *N*-acetylcysteine (NAC). The cells treated with the oxidant *tert*-butyl hydroperoxide (TBHP) (red) show increased staining with the CellROX Deep Red Reagent compared with cells pretreated with NAC before TBHP treatment (blue) and with untreated control cells (green). (**B**, **C**) CellROX Deep Red Reagent can be used with SYTOX Blue Dead Cell Stain to differentiate live stressed cells from dead cells. Jurkat cells were treated with (**B**) PBS or (**C**) 200 µM TBHP for 30 minutes before labeling using the CellROX Deep Red Flow Cytometry Assay Kit. Note that the treated cells (**C**) have a higher percentage of cells under oxidative stress than the basal level of ROS observed in control cells (**B**).

pHrodo *E. coli* BioParticles Phagocytosis Kits

- Specifically detect and monitor phagocytosis and endocytosis
- Track antibody internalization with pHrodo conjugates
- Track ligand internalization with pHrodo conjugates
- Combine with red or green dyes for multiplexed experiments

Proprietary, pH-sensitive Molecular Probes pHrodo dyes are almost nonfluorescent at neutral pH and fluoresce brightly in acidic environments, making them ideal for use as pH indicators for a variety of applications, including studying the processes of phagocytosis and endocytosis. The Invitrogen™ pHrodo™ Green *E. coli* BioParticles™ Phagocytosis Kit (Cat. No. P35381) and the Invitrogen pHrodo Red *E. coli* BioParticles Phagocytosis Kit (Cat. No. A10025; Figure 31) enable the detection of phagocytic activity in whole blood samples and live cell lines by flow cytometry. The kits include all of the reagents required for assessing particle ingestion and red blood cell lysis. Additionally, the Invitrogen pHrodo Red Phagocytosis Particle Labeling Kit (Cat. No. A10026) allows you to label your own sample of bacteria with pHrodo Red dye. To find more information and products, go to **thermofisher.com/flow-phrodo**

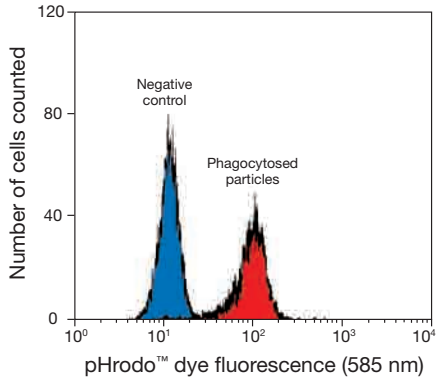


Figure 31. Flow cytometry analysis showing increased fluorescence of granulocytes treated with pHrodo Red BioParticles conjugates. A whole blood sample was collected and treated with heparin, and two 100 µL aliquots were prepared. Both aliquots were treated with pHrodo Red *E. coli* BioParticles conjugates (used in the pHrodo Red *E. coli* BioParticles Phagocytosis Kit, Cat. No. A10025) and vortexed. One sample was placed in a 37°C water bath, and the other sample (negative control) was placed in an ice bath. After a 15-minute incubation, red blood cells were lysed with an ammonium chloride–based lysis buffer. The samples were centrifuged for 5 minutes at 500 *x g*, washed once, and resuspended in Hank's Balanced Salt Solution. The samples were then analyzed on a Becton Dickinson FACSCalibur™ cytometer using a 488 nm argon laser and 564–606 nm emission filter. The sample incubated at 37°C shows the increased fluorescence of the phagocytosed pHrodo Red BioParticles conjugates (**red**), in contrast to the negative control sample, which was kept on ice to inhibit phagocytosis (**blue**).

Cell analysis product list

Section	Product name	Laser type	Ex/Em*	Regulatory status†	Size	Cat. No.
Cell viability	LIVE/DEAD Fixable Blue Dead Cell Stain Kit	UV	353/437	RUO	80 tests § 200 tests 400 tests §	L34961 L23105 L34962
	LIVE/DEAD Fixable Aqua Dead Cell Stain Kit	405	368/511	RUO	80 tests § 200 tests 400 tests §	L34965 L34957 L34966
	LIVE/DEAD Fixable Violet Dead Cell Stain Kit	405	416/541	RUO	80 tests § 200 tests 400 tests §	L34963 L34955 L34964
	LIVE/DEAD Fixable Yellow Dead Cell Stain Kit	405	405/552	RUO	80 tests § 200 tests 400 tests §	L34967 L34959 L34968
	LIVE/DEAD Fixable Green Dead Cell Stain Kit	488	495/521	RUO	80 tests § 200 tests 400 tests §	L34969 L23101 L34970
	LIVE/DEAD Fixable Red Dead Cell Stain Kit	488	583/603	RUO	80 tests § 200 tests 400 tests §	L34971 L23102 L34972
	LIVE/DEAD Fixable Far Red Dead Cell Stain Kit	633/635	651/672	RUO	80 tests § 200 tests 400 tests §	L34973 L10120 L34974
	LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit	633/635	753/783	RUO	80 tests § 200 tests 400 tests §	L34975 L10119 L34976
	LIVE/DEAD Fixable Dead Cell Stain Sampler Kit	Various	Various	RUO	320 assays	L34960
	SYTOX Blue Dead Cell Stain	UV/violet	444/480‡	RUO	1 mL	S34857
	SYTOX Green Dead Cell Stain	Blue	504/523‡	RUO	1 mL	S34860
	SYTOX Orange Dead Cell Stain	Yellow	547/570‡	RUO	1 mL	S34861
	SYTOX Red Dead Cell Stain	Red	640/658‡	RUO	1 mL	S34859
	SYTOX Dead Cell Stain Sampler Kit	Various	Various‡	RUO	1 kit	S34862
	SYTOX AADvanced Dead Cell Stain Kit	Blue	546/647‡	RUO	5 x 0.5 mL 0.2 mL	S10274 S10349
	Propidium Iodide ReadyProbes Reagent	Blue	535/617	RUO	6 × 2.5 mL	R37108
Cell proliferation	Click-iT Plus EdU Pacific Blue Flow Cytometry Assay Kit	Violet	404/450	RUO	50 assays	C10636
	Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay Kit	Blue	495/519	RUO	50 assays 100 assays	C10632 C10633
	Click-iT Plus EdU Alexa Fluor 647 Flow Cytometry Assay Kit	Red	650/670	RUO	50 assays 100 assays	C10634 C10635
	CellTrace Violet Cell Proliferation Kit	Violet	405/450	RUO	180 assays	C34557
	CellTrace CFSE Cell Proliferation Kit	Blue	492/517	RUO	1 kit	C34554
	CellTrace Far Red Cell Proliferation Kit	Red	630/661	RUO	1 kit	C34564
Cell cycle	Vybrant DyeCycle Violet Stain	Violet	369/437‡	RUO	200 µL	V35003
	Vybrant DyeCycle Green Stain	Blue	506/534‡	RUO	400 µL	V35004
	Vybrant DyeCycle Orange Stain	Blue	519/563‡	RUO	400 µL	V35005
	Vybrant DyeCycle Ruby Stain	Red	638/686‡	RUO	100 assays 400 assays	V10309 V10273
	FxCycle Violet Stain	Violet	358/461	RUO	500 assays	F10347
	FxCycle PI/RNase Staining Solution	Blue	535/617	RUO	100 mL	F10797
	FxCycle Far Red Stain	Red	640/658	RUO	500 assays	F10348

*Excitation and emission maximum wavelengths, in nm.
†RUO, For Research Use Only. Not for use in diagnostic procedures, unless otherwise indicated.
‡Excitation/emission wavelengths (in nm) when bound to DNA.
§Product size not available in all countries. Visit **thermofisher.com/livedead** to see what is available in your region.

Section	Product name	Laser type	Ex/Em*	Regulatory status†	Size	Cat. No.
Apoptosis	CellEvent Caspase-3/7 Green Flow Cytometry Assay Kit	Blue/ green	511/533 (CellEvent Green)‡ 546/647 (SYTOX AADvanced stain)‡	RUO	100 assays	C10427
	Annexin V, Pacific Blue conjugate	Violet	410/455	RUO	500 µL	A35122
	Annexin V, FITC conjugate	Blue	494/518	RUO	500 µL	A13199
	Annexin V, Alexa Fluor 488 conjugate	Blue	495/519	RUO	500 µL	A13201
	Annexin V, PE conjugate	Blue	496, 546, 565/578	RUO	250 µL	A35111
	Annexin V, APC conjugate	Red	650/660	RUO	250 µL	A35110
	Annexin V, Alexa Fluor 647 conjugate	Red	650/665	RUO	500 µL	A23204
	Annexin V Binding Buffer (5X)	NA	NA	RUO	50 µL	V13246
	Pacific Blue Annexin V/SYTOX AADvanced Apoptosis Kit	Violet/ blue	410/455 Pacific Blue dye 546/647 (SYTOX AADvanced stain)	RUO	50 assays	A35136
	Dead Cell Apoptosis Kit with Alexa Fluor 488 Annexin V & Propidium Iodide	Blue	495/519 (Alexa Fluor 488 dye) 535/617 (PI)	RUO	50 assays	V13241
					250 assays	V13245
	Dead Cell Apoptosis Kit with FITC annexin V & Propidium Iodide	Blue	494/519 (FITC) 535/617 (PI)	RUO	50 assays	V13242
	Violet Ratiometric Asymmetry Probe/Dead Cell Apoptosis Kit	Violet/ blue	405/530, 585 (F2N12S) 546/647‡ (SYTOX AADvanced stain)	RUO	100 assays	A35137
	MitoProbe JC-1 Assay Kit	Blue	514/529, 590§	RUO	100 assays	M34152
Other cell function assays	CellROX Green Flow Cytometry Assay Kit	Blue	508/525 (CellROX Green) 640/658 (SYTOX Red)‡	RUO	100 assays	C10492
	CellROX Orange Flow Cytometry Assay Kit	Green (532 nm)	545/565 (CellROX Orange) 640/658 (SYTOX Red)‡	RUO	100 assays	C10493
	CellROX Deep Red Flow Cytometry Assay Kit	Red	640/665 (CellROX Deep Red) 444/480 (SYTOX Blue)‡	RUO	100 assays	C10491
	pHrodo Green *E. coli* BioParticles Phagocytosis Kit	Blue	509/533	RUO	100 assays	P35381
	pHrodo Green *S. aureus* BioParticles Phagocytosis Kit	Blue	509/533	RUO	100 assays	P35382
	pHrodo Red *E. coli* BioParticles Phagocytosis Kit	Blue	560/585	RUO	100 assays	A10025
	pHrodo Phagocytosis Particle Labeling Kit	Blue	560/585	RUO	100 assays	A10026

*Excitation and emission maximum wavelengths, in nm.
†RUO, For Research Use Only. Not for use in diagnostic procedures unless otherwise indicated.
‡Excitation/emission wavelengths (in nm) when bound to DNA.
§Emission shift occurs with the concentration-dependent formation of red-fluorescent J-aggregates.

The Attune NxT flow cytometer

The Invitrogen™ Attune™ NxT Cytometer is a benchtop analyzer (Figure 32) that uses acoustic focusing—a revolutionary technology that precisely aligns cells using ultrasonic waves prior to interrogation with one or more lasers. This technology enables multicolor flow cytometric analysis with significantly greater collection rates and an improved ability to detect rare events without excess sample manipulation. The Attune NxT system offers:

- Unique modular design—one-day field-upgradable system with configurations for 1–4 lasers and up to 14 emission channels
- Fast detection speeds—short acquisition times without loss of data quality enable applications such as rare-event detection and use of no-lyse/no-wash techniques
- Distinctive acquisition and analysis software—intuitive and powerful for users of all experience levels
- Convenient size—complete setup fits on your benchtop

With up to 4 lasers and 14 emission channels for multiparameter analysis, the Attune NxT flow cytometer was designed as a modular system to fit most experimental needs and lab budgets (Table 14). The novel design of the optical path helps ensure precise fixed alignment of 4 spatially separated lasers onto the sample stream, offering consistency in data over time, superior performance and excellent reliability. Minimal compensation is required for popular dye combinations (Figures 33 and 34).

Whether you configure your system now or upgrade later, the Attune NxT flow cytometer can grow with you and your research needs. To find out more and request an in-lab demonstration, visit **thermofisher.com/attune**

Figure 32. The Attune NxT Flow Cytometer

Table 14. Specifications for the Attune NxT cytometer.

Product name	Attune NxT flow cytometer
Size, weight	40 x 58 x 43 cm (16 x 23 x 17 in), 29 kg (64 lb)
Electrical	100–240 VAC, 50/60 Hz, <150 W
Excitation	Up to 4 lasers: Violet (405 nm, 50 mW), blue (488 nm, 50 mW), yellow (561 nm, 50 mW), red (637 nm, 100 mW); Laser profile: Flat-top laser requiring minimal alignment; Flow cell: Quartz cuvette gel-coupled to 1.2 NA collection lens
Emission	Forward scatter: Photodiode detector with 488/10 bandpass filter; Side scatter: PMT with 488/10 bandpass filter; Emission filters: User-changeable, keyed filters
Fluidics	Sample rate: 12.5–1,000 µL/min; Sample analysis volume: 20 µL–4 mL; Sample tube size: 17 x 100 mm to 8.5 x 45 mm; Sample delivery: Positive-displacement syringe pump; Fluid storage: All fluids stored within instrument with active fluid level sensing (optional external tank available)
Performance	Data acquisition rate: Up to 35,000 events/sec; Maximum event file: 20 million; Particle size range: 0.5–50 µm; Automated maintenance: ≤15 min startup and shutdown

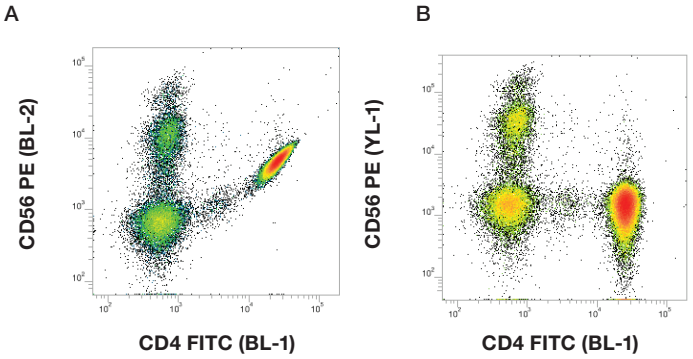
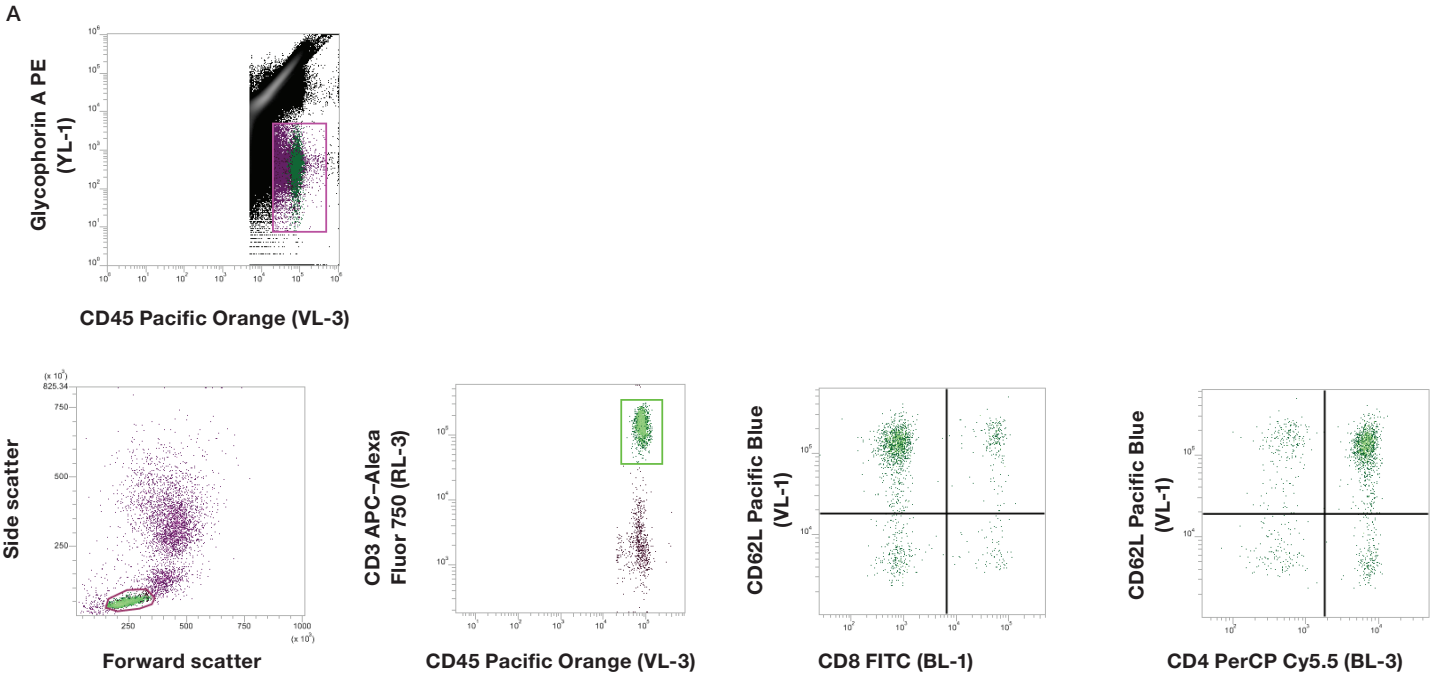


Figure 33. With the Attune NxT flow cytometer, minimal compensation is required for popular dyes such as FITC and PE. (**A**) When FITC and PE use the same excitation and optical detection pathways after excitation by the 488 nm blue laser, there is a significant amount of spillover of FITC signal into the PE detector, requiring compensation. (**B**) When excitation and detection of FITC and PE are uncoupled, using the 561 nm laser to excite PE and the 488 nm laser to excite FITC, there is little or no spillover of FITC signal into the PE signal, thus no compensation is required.



B

Compensation Matrix

	Pacific Blue™ (VL-1)	Pacific Orange™ (VL-3)	FITC (BL-1)	PerCP Cy®5.5 (BL-3)	PE (YL-1)	APC Alexa Fluor™ 750 (RL-3)
Pacific Blue™ (VL-1)		0.0	0.0	0.0	0.0	0.0
Pacific Orange™ (VL-3)	0.0		0.0	0.0	0.0	0.0
FITC (BL-1)	0.0	0.0		0.0	0.0	0.0
PerCP Cy®5.5 (BL-3)	0.0	0.0	0.0		0.0	0.0
PE (YL-1)	0.0	0.0	0.0	0.0		0.0
APC Alexa Fluor™ 750 (RL-3)	0.0	0.0	0.0	0.0	0.0	

Figure 34. Minimize compensation for multicolor panels. (**A**) Optimal design of a no-lyse, no-wash 6-color immunophenotyping panel for human T cell subsets acquired on the Attune NxT flow cytometer requires no compensation. Whole human blood was stained with anti-CD45 Pacific Orange (Cat. No. MHCD0327), anti–glycophorin A PE (Cat. No. MHGLA04), anti-CD3 APC–Alexa Fluor 750 (Cat. No. MHCD0327), anti-CD62L Pacific Blue (Cat. No. MHCD0828), anti-CD8 FITC (Cat. No. MHCD0801) and anti-CD4 PerCP-Cy5.5 (Cat. No. A15858) antibodies. Samples were acquired on the Attune NxT Flow Cytometer using 405 nm excitation to detect Pacific Blue and Pacific Orange dyes, 488 nm excitation to detect FITC and PerCP-Cy5.5, 561 nm excitation to detect PE and 637 nm excitation to detect APC–Alexa Fluor 750. A fluorescence threshold was set on the CD45–Pacific Orange conjugate and red blood cell–coincident events were excluded based on glycophorin A–PE positivity. Lymphocytes were gated based on scatter properties, from which T cells were identified by CD3 expression. T cells were then analyzed for their expression of the lineage markers CD4 and CD8 and the activation marker CD62L to identify naive/central memory T cells ($CD62L^+$) and effector memory T cells ($CD62L^-$). (**B**) Compensation matrix from the Attune NxT flow cytometer for the 6-color immunophenotyping panel. Zero compensation is needed.

What is acoustic focusing?

The Attune NxT Flow Cytometer uses ultrasonic waves (over 2 MHz, similar to those used in ultrasound medical imaging), in combination with hydrodynamic forces, to position cells into a single, focused line along the central axis of a capillary (Figure 35). Acoustic focusing is largely independent of the sample input rate, enabling cells to be tightly focused at the point of laser interrogation, regardless of the sample-to-sheath ratio. This, in turn, allows the collection of more photons for high-precision analysis at superior volumetric sample throughput, as demonstrated by the minimal variation demonstrated in cell cycle analysis (Figure 36). Note that the sample input rates range from 12.5 µL/min to 1 mL/min, up to 10 times faster than traditional hydrodynamic focusing systems.

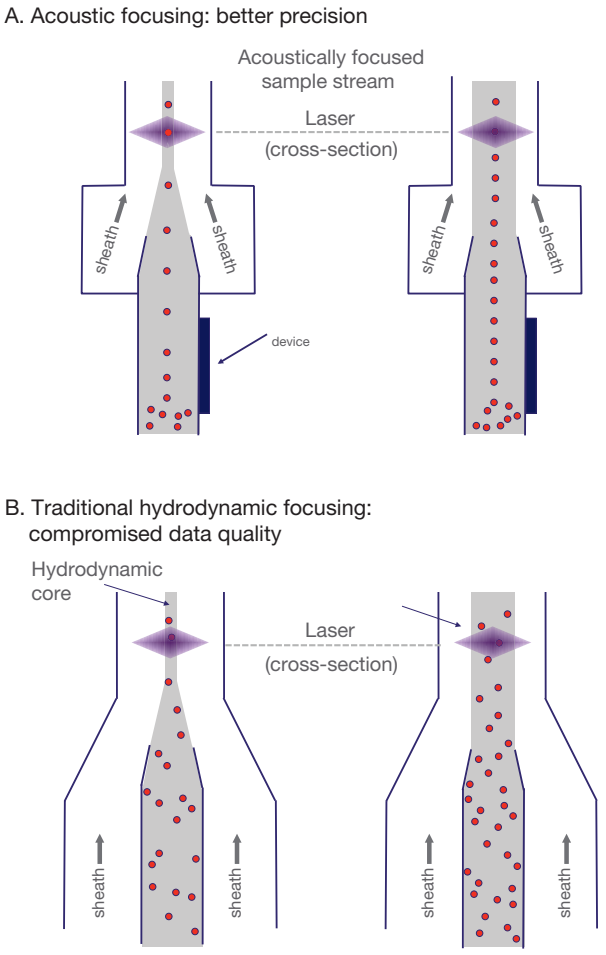
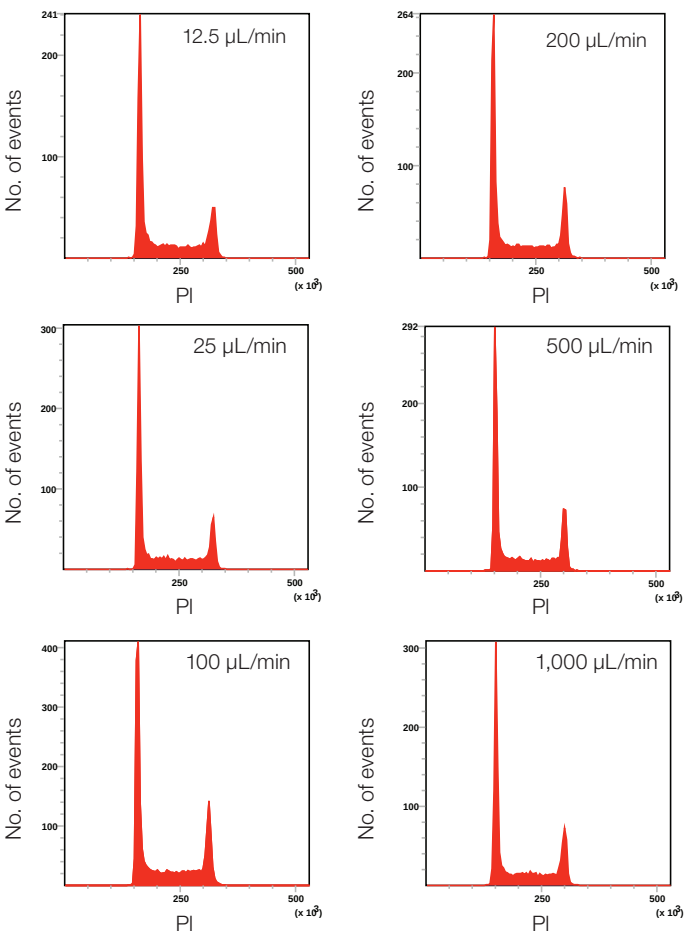


Figure 35. Acoustic focusing vs. traditional hydrodynamic focusing as particles pass through the laser. (**A**) In acoustic focusing, cells remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. (**B**) In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality.



Sample rate (µL/min)	12.5	25	100	200	500	1,000
G_0/G_1 CV (%)	2.99	3.03	2.76	2.94	2.70	2.96
G_2/M CV (%)	1.99	1.99	1.99	2.05	2.05	2.03

Figure 36. Minimal data variation at high sample rates with the Attune NxT flow cytometer. Jurkat cells were fixed and stained with propidium iodide, treated with RNase and analyzed at a concentration of 1 x 10^6 cells/mL on the Attune NxT flow cytometer at different sample rates. The left peak in all graphs reflects cells in G0/G1 phase, while the right peak reflects cells in G2/M phase. Regardless of sample rate, the width of the G0/G1 and G2/M peaks and coefficient of variation (CV) remains consistent for the Attune NxT flow cytometer, even at the highest sample rate of 1,000 µL/min.

The Attune NxT Autosampler

An optional accessory for the Attune NxT Flow Cytometer, the Invitrogen™ Attune™ NxT Autosampler enables the rapid processing of multiple samples. Key features include:

- Compatible with many different plate formats, including 96-well, 384-well and deep-well plates (Figure 37)
- Intelligent probe design minimizes clogging and carryover (<1%) (Table 15) and helps prevent damage to the instrument
- Mixes sample by aspiration to help maintain sample homogeneity and cell viability (Table 16)
- Performs automated cleaning when the instrument is shut down
- Helps maintain consistent data while easily switching between use of the autosampler and individual tubes
- Consistent data across different collection rates

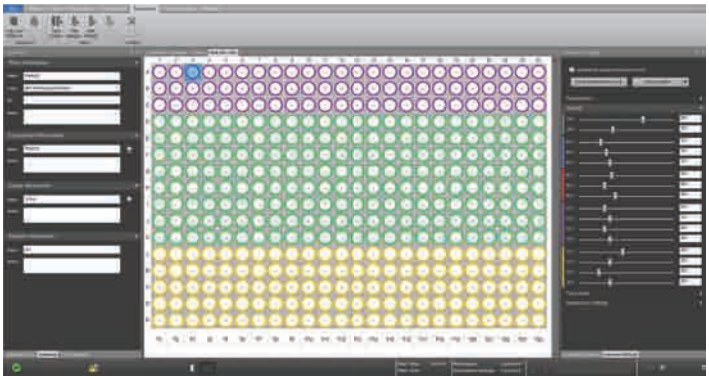


Figure 37. Example of a 384-well heat map.

Table 15. Minimal carryover using the Attune NxT Autosampler. Jurkat cells at a concentration of 1 x 10^6 cells/mL were dispensed into a 96-well V-bottom plate and sampled using the Attune NxT Autosampler. Samples were analyzed on the Attune NxT flow cytometer using collection rates in Standard mode (200 µL/min) and High Throughput mode (500 µL/min). Each sample was mixed once and the Attune NxT Autosampler was washed 1–3 times prior to sampling the next well. Percent sample carryover was calculated.

	Number of washes and % carryover		
Mode	1	2	3
Standard	0.01	0.01	0.01
High Throughput	0.02	0.02	0.02

Table 16. Gentle sample mixing using the Attune NxT Autosampler: increasing the number of mixing cycles does not adversely affect cell viability. Ammonium chloride–lysed whole blood (LWB) and NIH/3T3 (live/heat-treated) cells were stained with 2 µg/mL propidium iodide and loaded in triplicate into a 96-well V-bottom plate. Prior to acquisition samples were mixed 0–5 times by the Attune NxT Autosampler and then samples were analyzed using Standard mode collection rates (100 µL/min for NIH/3T3, 200 µL/min for LWB) on the Attune NxT flow cytometer. Propidium iodide was excited using a 488 nm laser and fluorescence emission was collected using a 640 nm longpass emission filter. Minimal variation was observed within each cell type, regardless of the number of mix cycles used prior to acquisition.

	Number of washes and % carryover	
Number of mix cycles	LWB	NIH/3T3
0	0.75	34.10
1	0.78	32.83
2	0.74	33.52
3	0.74	32.75
4	0.74	33.26
5	0.75	31.58

Attune NxT Flow Cytometer product list

Product List	No. of lasers	No. of emission channels *	Cat. No.
Attune NxT Flow Cytometer—Blue Laser	1	4	A24864
Attune NxT Flow Cytometer—Blue/Red Lasers	2	7	A24863
Attune NxT Flow Cytometer—Blue/Violet Lasers	2	8	A24862
Attune NxT Flow Cytometer—Blue/Yellow Lasers	2	7	A24861
Attune NxT Flow Cytometer—Blue/Red/Violet Lasers	3	11	A24860
Attune NxT Flow Cytometer—Blue/Violet/Yellow Lasers	3	11	A24859
Attune NxT Flow Cytometer—Blue/Red/Violet/Yellow Lasers	4	14	A24858
Attune NxT Autosampler—without computer	NA**	NA	4473928

*Number of different emission channels, in addition to the forward scatter (FSC) and side scatter (SSC) detection channels.
**NA, not applicable

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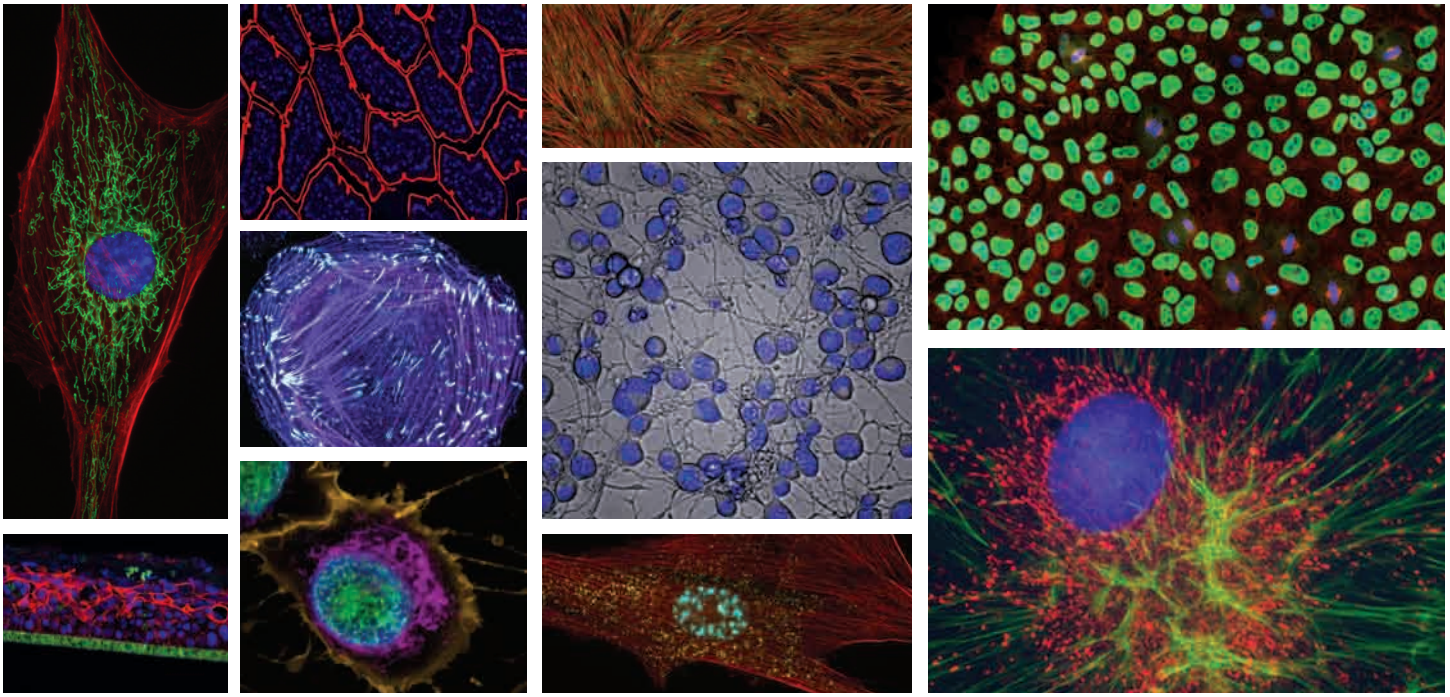

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