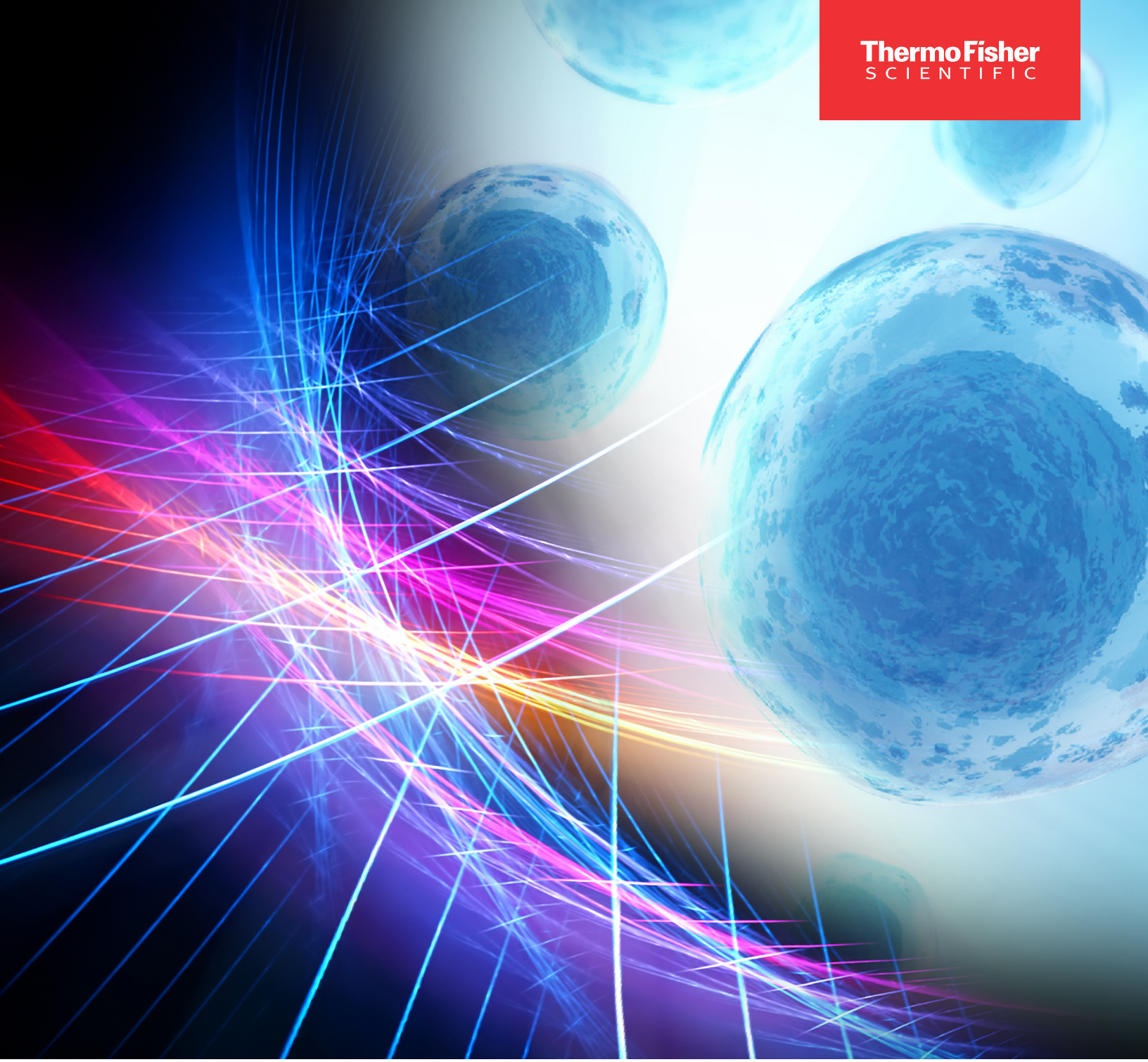


Flow cytometry and cell sorting capabilities guide

For conventional and spectral flow cytometry

Complete flow cytometry solutions—trusted for speed, accuracy, and precision

invitrogen

Getting started

Flow cytometry enables the simultaneous analysis of multiple proteins, gene expression, and cell functions such as oxidation, viability, cell cycle, apoptosis, and proliferation from an individual cell. This technology makes it possible to obtain a statistically relevant amount of data by combining information from individual cells to gain insight into a heterogeneous sample. Whether you are identifying cell subpopulations or investigating cell functions, flow cytometry can make significant contributions to moving your research forward.

Building a flow cytometry experiment often requires combining products into a multicolor panel. Use this guide to understand the basics of Invitrogen™ eBioscience™ flow cytometry antibodies and Invitrogen™ flow cytometry assays and reagents. Then see how example panels are run on flow cytometers, including Invitrogen™ Attune™ flow cytometers, in the following areas:

- Immunology
- Inflammation
- Immuno-oncology
- Solid-tumor cancers
- Neuroinflammation
- Gene editing
- Microbiology

Flow cytometry workflow—what you will need

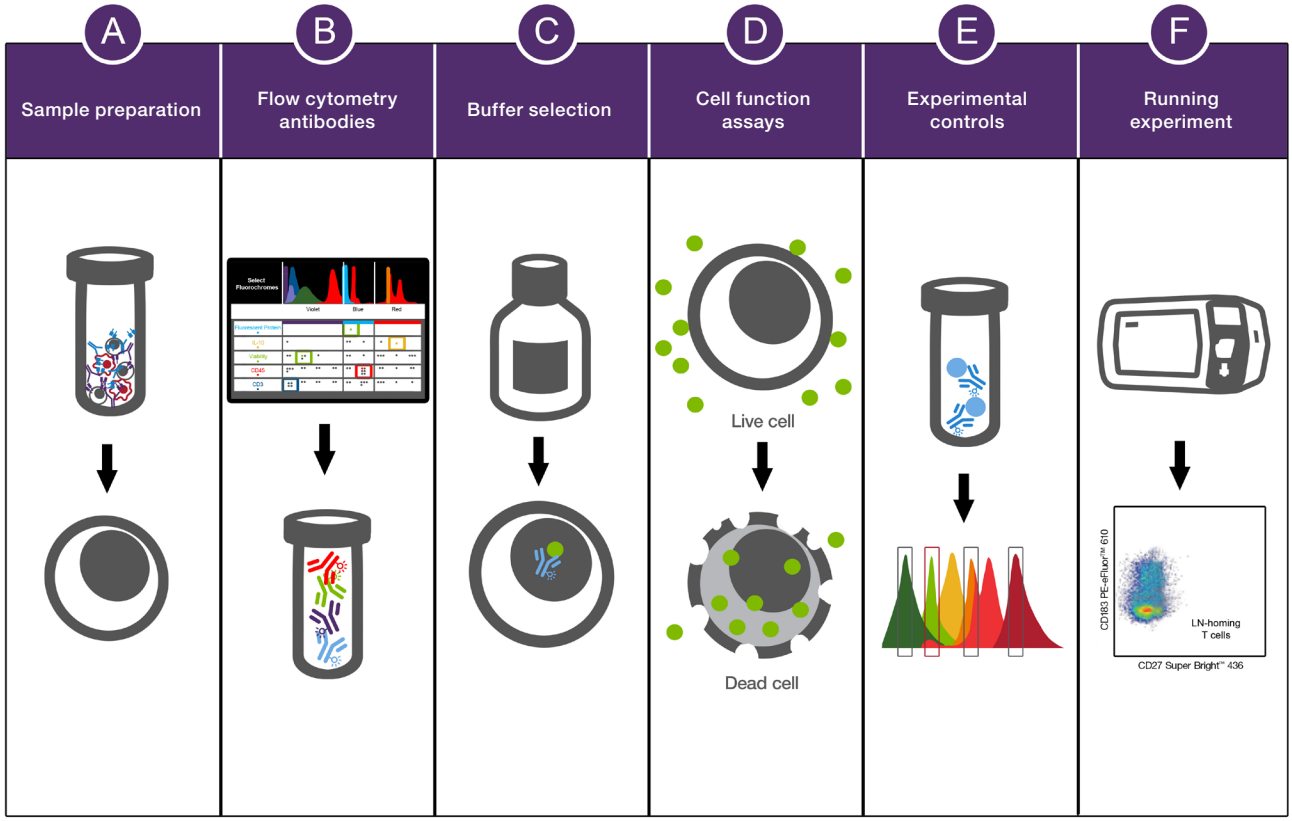


Figure 1. Flow cytometry workflow. Planning your workflow in advance as outlined will help generate a successful experiment.

Find out more about multipurpose flow cytometry experiments at thermofisher.com/flowcytometry

Sample preparation: reagents for immune cell activation

Stimulation or treatment of cells is usually required for activation of immune cells to proliferate and differentiate into mature cell types (Figure 2). Activated cells often express higher levels of transcription factors, cytokines, chemokines, and other mediators detected by flow cytometry. Choosing the appropriate activating reagent will depend on (1) cell type, (2) expression and kinetics of the protein of interest, and (3) experimental conditions.

We offer an expansive list of high-quality cell stimulation products that include:

- Functional-grade antibodies and recombinant proteins to stimulate many types of immune cells
- Reagents in appropriate preservative-free buffers with low endotoxin levels to use in cell culture
- The Invitrogen™ eBioscience™ Cell Stimulation Cocktail at a ready-to-use concentration

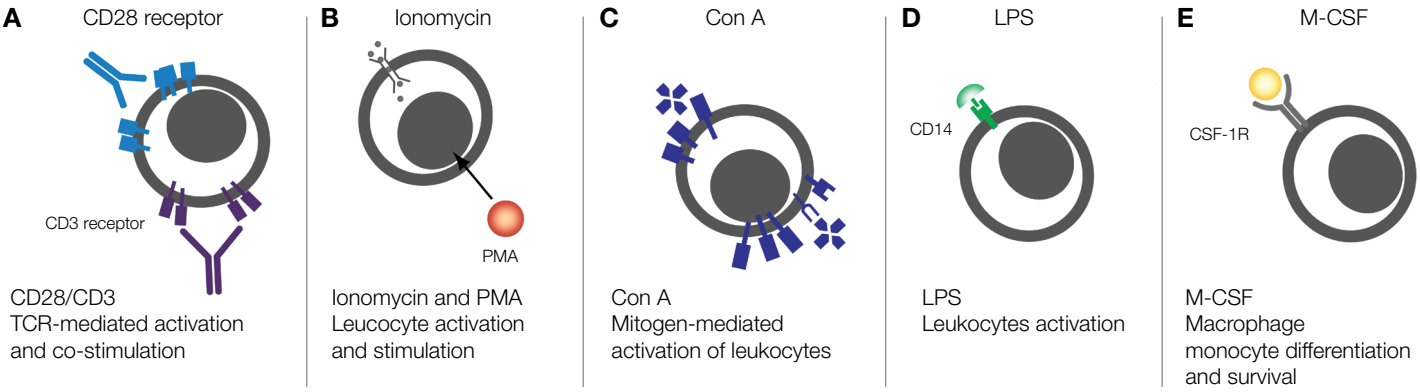


Figure 2. Cell stimulation reagents. (A) Functional-grade antibodies (e.g., anti-CD3 and anti-CD28) or Invitrogen™ Dynabeads™ magnetic beads for T cell activation and expansion. **(B)** eBioscience Cell Stimulation Cocktail comprising phorbol 12-myristate 13-acetate (PMA), a protein kinase activator, and ionomycin, a calcium ionophore, stimulates T cells to produce interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-2 (IL-2), and interleukin-4 (IL-4). **(C)** Concanavalin A (Con A) induces T cell activation and proliferation. **(D)** Monocytes can be activated by lipopolysaccharide (LPS) to secrete interleukin-6 (IL-6), interleukin-10 (IL-10), or TNF-α. **(E)** Macrophage colony-stimulating factor (M-CSF) is a growth factor that regulates the proliferation, differentiation, and functional activation of monocytes' differentiation into macrophages.

Example: T cell activation

T cells require external signals for differentiation and expansion from a quiescent state (Figure 3). PMA and ionomycin or anti-CD3 and anti-CD28 antibodies are recommended to upregulate intracellular transcription factors for detection. Time-course profiling of cells with the cell-stimulating reagents is recommended, since cytokines have different kinetics and/or expression levels.

Identification of human Th1 cells within a CD4⁺ T cell population

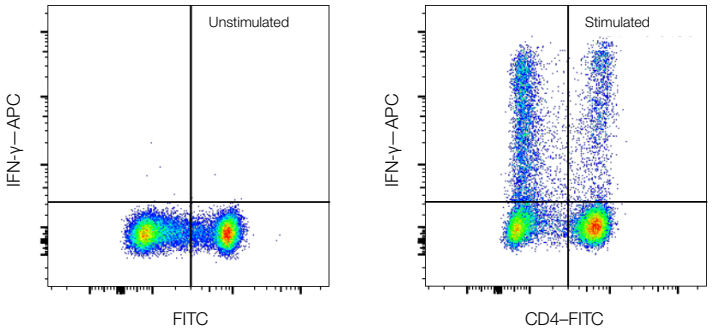


Figure 3. Identification of human Th1 cells within a CD4⁺ T cell population. Normal human peripheral blood cells were unstimulated (left) or stimulated with eBioscience Cell Stimulation Cocktail plus protein transport inhibitors (500X) (right). Cells were fixed and stained intracellularly with Invitrogen™ anti–human CD4 FITC and anti–human IFN-γ APC, using the Invitrogen™ eBioscience™ Intracellular Fixation and Permeabilization Buffer Set and protocol. Cells in the lymphocyte gate were used for analysis.

Find out more at thermofisher.com/flowreagents

Flow cytometry antibodies

A multicolor flow cytometry panel uses two or more primary conjugated antibodies to identify single cells by detecting multiple antigens. The goal of the panel is to get the maximum signal for effective visualization of cell populations. Use this section of the guide to aid in the selection of antibodies.

Flow cytometry antibodies cover:

- CD markers
- Transcription factors
- Cytokines, chemokines, and growth factors
- Signaling pathway markers, including phosphoproteins

Marker selection

Choose from our selection of more than 80 fluorophore dye options and more than 28,000 flow cytometry antibodies (more than 1,500 Invitrogen™ Brilliant Ultra Violet™ and Brilliant Violet™ antibodies). Each flow cytometry antibody search result contains data plots gathered from internal antibody validation* testing and published customer data accessible online. Use this online search tool to determine which antibody is applicable to find your cell population (Figure 4).

Our flow cytometry antibodies are conjugated to different fluorophores to allow for use on any instrument. These fluorophores simplify the optimization of panel design because of flexible dye selection for reduced spectral overlap.

Choose dyes based on:

- Laser and filter configuration of the flow cytometer
- Expression level or abundance of the target protein
- Fluorophore brightness
- Fluorescence excitation emission spectra

Example: selecting the right fluorophore

Fluorophore selection is important for finding your cell of interest. Pick fluorophores with less spectral overlap to clearly identify two populations (Figure 5). Match brighter fluorophores with less abundant targets, and dimmer fluorophores with abundant targets for greater signal separation.

* The use or any variation of the word "validation" refers only to research-use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

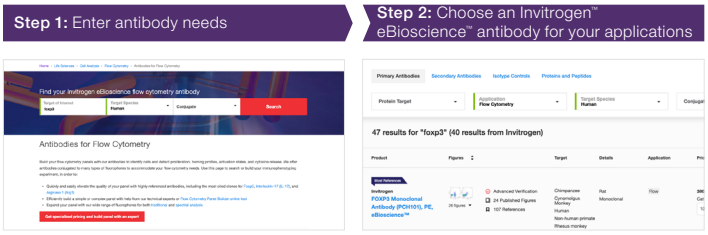


Figure 4. Antibody search tool to find information and purchase antibodies. Antibody application data from customer publications and internal testing data (left). A list of antibodies can be purchased, or saved and shared for later use (right).

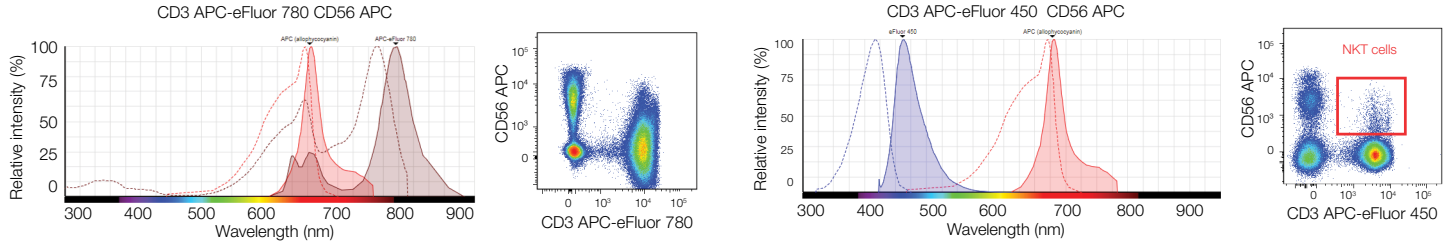


Figure 5. Normal human peripheral blood cells were stained with anti–human CD3 antibody conjugated with Invitrogen™ eBioscience™ APC-eFluor™ 780 dye (left) or APC-eFluor™ 450 dye (right), as well as anti–human CD56 antibody conjugated with APC dye. Cells in the lymphocyte gate were used for analysis.

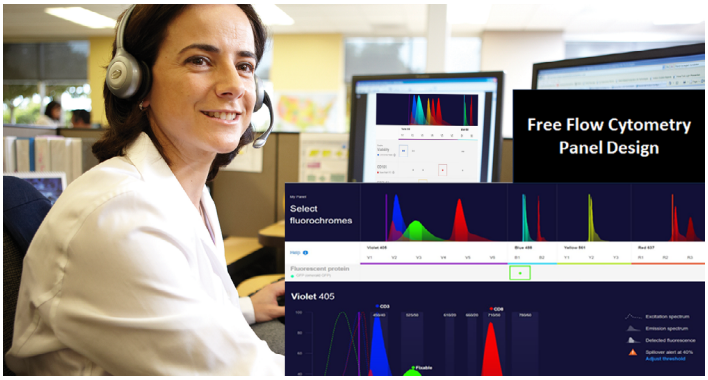
Find your flow cytometry antibodies at **thermofisher.com/flowantibodies**

Table 1. Comprehensive list of available fluorophores based on their usage, benefits, and intended applications.

Family	Type	Benefit	Invitrogen™ fluorophore
DNA-scaffold dyes—recent dye innovation	Invitrogen™ eBioscience™ NovaFluor™ dyes	• Unique spectral signatures • Minimal compensation and spectral spread • Low cross-laser excitation • Named for the excited laser and emission spectrum • Use Invitrogen™ CellBlox™ Blocking Buffer (Cat. No. B001T03F01) for use with NovaFluor dyes to label cells to block nonspecific labeling and to reduce background	NovaFluor UV (Ultra Violet) 765* dye NovaFluor Violet 690,* 745, and 800* dyes NovaFluor Blue 510, 530, 555, 585, 610-30S, 610-70S , 660-40S, 660-120S, 690, 725,* 745, 760, and 800 dyes NovaFluor Yellow 570, 590, 610, 660, 690, 700, 730, 755, and 810 dyes NovaFluor Red 660, 685, 700, 710, 725, 755, and 800 dyes Learn more about the dye selection at thermofisher.com/novafluor
Polymer dyes—recent dye innovation	Invitrogen™ eBioscience™ Brilliant Violet™ and Brilliant Ultra Violet™ dyes	• Excited by the violet and ultra violet lasers • Add Invitrogen™ Brilliant Stain Buffer (Cat. No. 00-4409-75) when using two or more polymer dyes to lower background levels	Brilliant Ultra Violet 395, 496, 563, 615, 661, 737, and 805 dyes Brilliant Violet 421, 480, 605, 650, 711, and 786 dyes Learn more about dye selection at thermofisher.com/brilliantdyes
Polymer dyes	Invitrogen™ eBioscience™ Super Bright dyes and their tandems	• Excited by the 405 nm violet laser • Minimal spillover into other channels • Add Invitrogen™ eBioscience™ Super Bright Complete Staining Buffer (Cat. No. SB-4401-42) when using two or more polymer dyes to lower background levels	Super Bright 436, 600, 645, 702, and 780 dyes
Large, protein-based molecules	Original	• Cost-efficient • Some of the brightest dyes available	APC (allophycocyanin) PerCP (peridinin chlorophyll protein) PE (phycoerythrin)
	Tandem dyes	• Dyes occupy different channels from the donor molecule, and this can be used to build larger panels	APC-Cyanine5 and 7 dyes PE-Alexa Fluor 610 and 700 dyes APC-Alexa Fluor 750 dye APC-eFluor 780 dye PE-Cyanine5 (TRI-COLOR dye), 5.5, and 7 dyes PE-Texas Red dye PE-eFluor 610 dye PerCP-Cyanine5.5 dye PerCP-eFluor 710 dye
Organic dyes—small, stable molecules	Original	• Cost-efficient	FITC
	Invitrogen™ Pacific™ dyes	• Some of the dimmest dyes	Pacific Blue™ dye Pacific Orange™ dye
	Invitrogen™ Alexa Fluor™ dyes	• Photostable dyes that range across the visible spectrum • Used in flow cytometry and imaging • Named for their excitation wavelengths	Alexa Fluor 405, 488, 532, 561, 647, 660, and 700 dyes
	Invitrogen™ eBioscience™ eFluor™ organic dyes	• Engineered for detection for flow cytometry • Named for their emission wavelength	eFluor 450, 506, and 660 dyes
Nanocrystals	Invitrogen™ Qdot™ dyes	• Narrow emission • Large Stokes shift	Qdot 605, 655, 705, and 800 dyes

* This dye fits in a spectral space that's currently inaccessible to other dyes.

Free: Flow cytometry Panel Design Service and Panel Builder Tool



Key features of our free Panel Design Service include:

- **Free and fast**—no purchase necessary; typical response time within one business day
- **Personalized**—one-on-one, customized assistance with a live specialist
- **Flexible**—accommodates antibodies that you already have, and those that you need

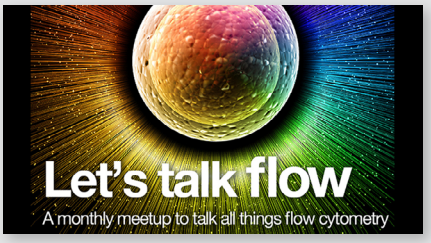
Panel Builder Tool—for self-designing panels

The flow cytometry Panel Builder Tool is a free online tool to help select antibody conjugates and reagents for a multicolor flow cytometry panel. This allows for improved panel design with greater separation and detection of individual cell populations of interest.

With this tool, you can:

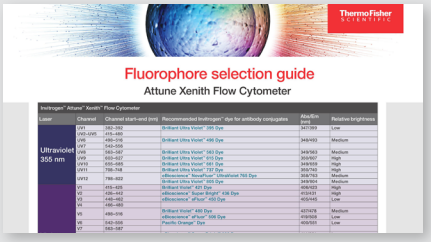
- Create a new immunophenotyping panel or update an existing one—start fresh, customize a preconfigured panel, or bulk-load antigens for faster selection
- Check fluorophore emission spectra with the built-in SpectraViewer
- Export an Excel™ document with your antibody choices, or order directly

Step 1: Cytometer → **Step 2:** Antigens → **Step 3:** Panel builder → **Step 4:** Products → **Step 5:** Summary



Flow cytometry monthly webinar series —Let's talk flow
"Let's talk flow" is a bite-sized education platform that helps provide exclusive face-to-face time with industry leaders, helping you enhance your flow cytometry knowledge and skills.

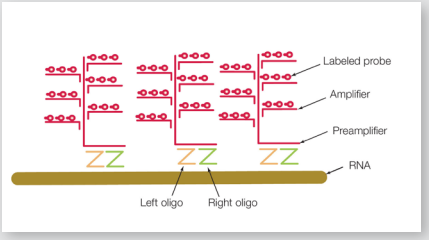
Register once for lifetime access at thermofisher.com/letstalkflow



Download fluorophore and reagent selection guides
Find recommended flow cytometry antibodies and reagents for your flow cytometers. Download guides for Invitrogen™ Attune™ Xenith™ Flow Cytometer, BD FACSDiscover™ S8 Sorter, Cytek™ Aurora Cell Sorter, and more.

- Attune Xenith Flow Cytometer
- SONY ID7000™ Spectral Cell Analyzer
- BD FACSDiscover S8 Sorter
- Agilent NovoCyte™ Penteon
- Cytek Aurora/CS
- BD FACSymphony™ A5/A5 SE

Visit thermofisher.com/flowantibodies



Want to detect RNA by flow cytometry, especially when antibodies for immunophenotyping are unavailable?
The Invitrogen™ PrimeFlow™ RNA Assay expands the capabilities of flow cytometry to include RNA detection. By combining the PrimeFlow assay with immunolabeling of both cell surface and intracellular proteins using fluorophore-conjugated antibodies, researchers can simultaneously analyze RNA transcription and protein expression patterns at single-cell resolution.

Learn more at thermofisher.com/primeflow

For panel design service and panel builder tool visit **thermofisher.com/paneldesign**

Buffer selection: fixation and permeabilization reagents

Fixatives are necessary for saving samples to be used later or for looking at intracellular or intranuclear targets. Ready-to-use fixation kits are optimized for flow cytometry applications. Benefits of using these kits include the following:

- Methods used to stain cells take into consideration the location of the target proteins
- The fixation and permeabilization procedure keeps the morphological light-scattering characteristics of the cells intact
- The reagents in the kits help reduce background staining

Table 2. Cell staining workflow.

	Cell-surface staining (CD markers)	Cytoplasmic staining (cytokines)	Nuclear and cytoplasmic staining (cytokines and transcription factors)
Stain surface proteins	✓	✓	✓
Fix cells		✓	✓
Permeabilize cells		✓	✓
Stain cytoplasmic proteins		✓	✓*
Stain nuclear proteins			✓

* Cytoplasmic proteins may be stained with a nuclear staining kit, but it may not be optimal.

Table 3. Flow cytometry buffer and reagent selection guide.

Staining buffer	Description	Location
eBioscience Flow Cytometry Staining Buffer	Cell-surface markers are often used to identify cell types. Permeabilization techniques can damage or denature cell-surface antigens and prevent antibodies from binding to surface epitopes. It is advisable to stain for cell-surface antibodies separately. Cell-surface markers can also be stained first, and then protocols for cytoplasmic or nuclear staining should be followed.	Cell surface
Invitrogen™ FIX & PERM™ Cell Permeabilization Kit or **Intracellular Fixation and Permeabilization Buffer Set**	Cytoplasmic proteins can include cytokines, organelles, and cytoplasmic transcription factors. These proteins are easily accessible with gentle fixation and light permeabilization. Fixation of cytoplasmic proteins often requires a crosslinking agent to have the protein trapped within the cell.	Cytoplasm
Invitrogen™ eBioscience™ Foxp3/Transcription Buffer Set	Transcription factors, DNA-binding proteins, and modified proteins make up the bulk of nuclear proteins. A quick fixation combined with a stringent permeabilization allows antibodies to penetrate into the nucleus. Fixation reagents can include either crosslinking agents or organic solvents. This type of protocol is also appropriate when examining proteins found both in the cytoplasm* and nucleus.	Nucleus

* Cytoplasmic proteins may be stained with a nuclear staining kit, but it may not be optimal.

Find out more about buffers at **thermofisher.com/flowbuffers**

Cell function assays: dyes and reagents

Flow cytometry is more than just panels with antibodies. Fluorophore reagents can be used to label cell functionalities such as viability and mitochondrial oxidation.

These reagents and assays can be incorporated into a flow cytometry panel just like a flow cytometry antibody. Use the chart below to determine which assays can be incorporated into a panel (Figure 6).

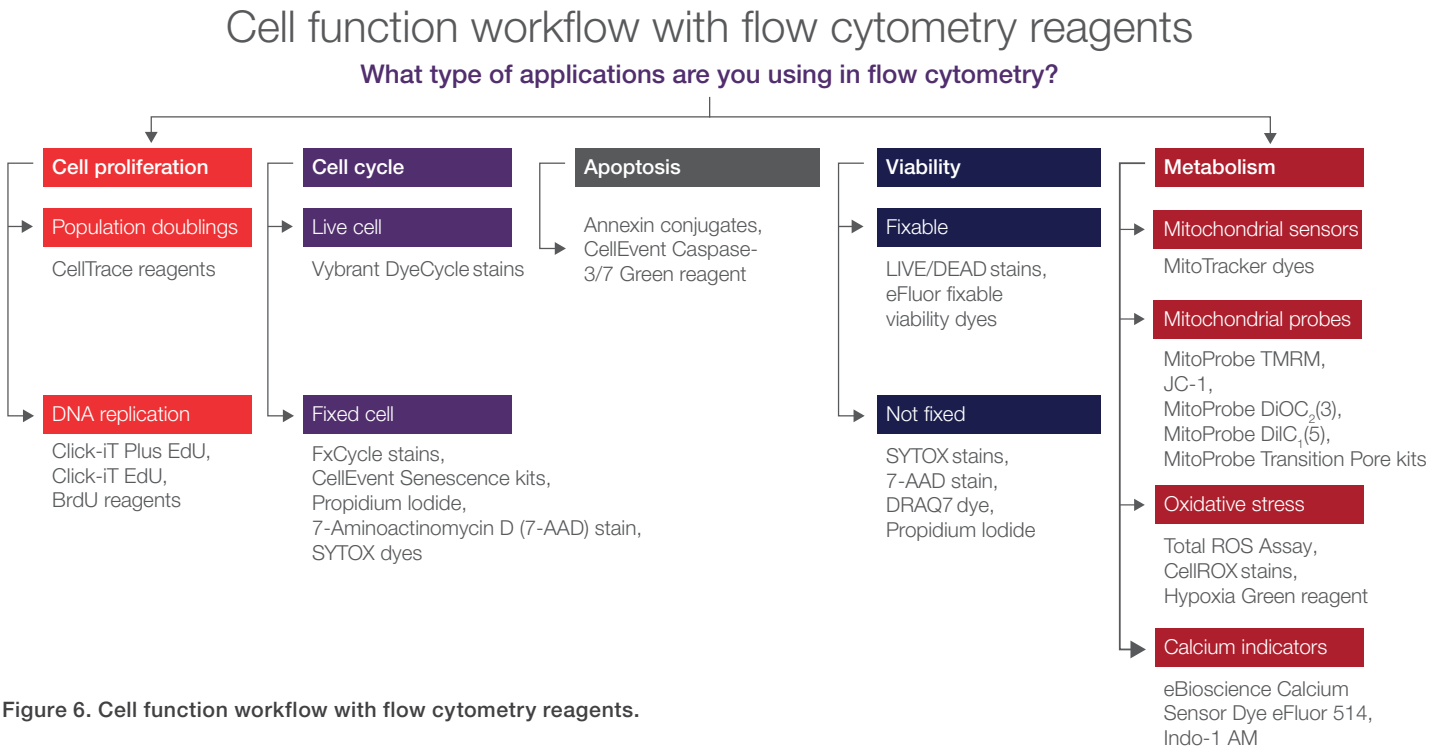


Figure 6. Cell function workflow with flow cytometry reagents.

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. Our assays are all one- or two-step processes and can be used in cell sorting or analysis applications.

Membrane dyes to characterize extracellular vesicles (EVs)

Uniformly label a population of EVs from cell culture. These reagents stain lipids, which is useful for EV detection.

- Lipophilic styryl dye: Invitrogen™ FM™ dye
- Long-chain lipophilic carbocyanine dyes: Invitrogen™ DiI, Vybrant™ CM-DiI (fixable), DiO, and DiD dyes, or Vybrant™ Multicolor Cell Labeling Kit
- Invitrogen™ Di-8-ANNEPS dyes

Table 4. Cell viability dye selection guide.

Laser	Nonfixable stains	Fixable stains
UV	DAPI (470)	LIVE/DEAD Fixable Blue (450)
405 nm	SYTOX Blue (480)	LIVE/DEAD Fixable Violet (451)
		LIVE/DEAD Fixable Lime (506)
		LIVE/DEAD Fixable Aqua (526)
		LIVE/DEAD Fixable Yellow (575)
488 nm	SYTOX Green (523)	LIVE/DEAD Fixable Green (520)
	Propidium Iodide (617)	LIVE/DEAD Fixable Olive (557)
	SYTOX AADvanced (647)	LIVE/DEAD Fixable Red (615)
561 nm	SYTOX Orange (570)	LIVE/DEAD Fixable Orange (602)
	SYTOX AADvanced (647)	
633/5 nm	SYTOX Red (660/20*)	LIVE/DEAD Fixable Far Red (665)
		LIVE/DEAD Fixable Scarlet (723)
		LIVE/DEAD Fixable Near IR (775)
		LIVE/DEAD Fixable Near IR (780)
808 nm		LIVE/DEAD Fixable Near IR (876)

* Emission maximum (nm).

Find out more at thermofisher.com/flowreagents

Example: avoiding inaccurate analysis with a LIVE/DEAD assay

When choosing a viability dye to stain cells after fixation, it is important to select one that is retained in the cell post-fixation to preserve the staining pattern. Excluding dead cells from the data allows cleaner separation and identification of cell populations. Brightfield images collected using the Invitrogen™ Attune™ CytPix™ Flow Cytometer confirm your gating to give you more confidence in your data (Figure 7). Invitrogen™ LIVE/DEAD™ fixable dead cell stains are fixable viability dyes that help you accurately assess the viability of cells in samples after fixation and/or permeabilization (Figure 8).

Application spotlight—bacterial cell viability workflow

Flow cytometry methods can shorten bacterial phenotyping and counting time.

- To obtain a single bacterial cell suspension, beverages and solid foods should be weighed and homogenized
- Serial dilution is not necessary—just take a stained sample, dilute, and analyze
- Invitrogen™ LIVE/DEAD™ *Bac*Light™ kits can be used to quickly determine bacterial cell viability

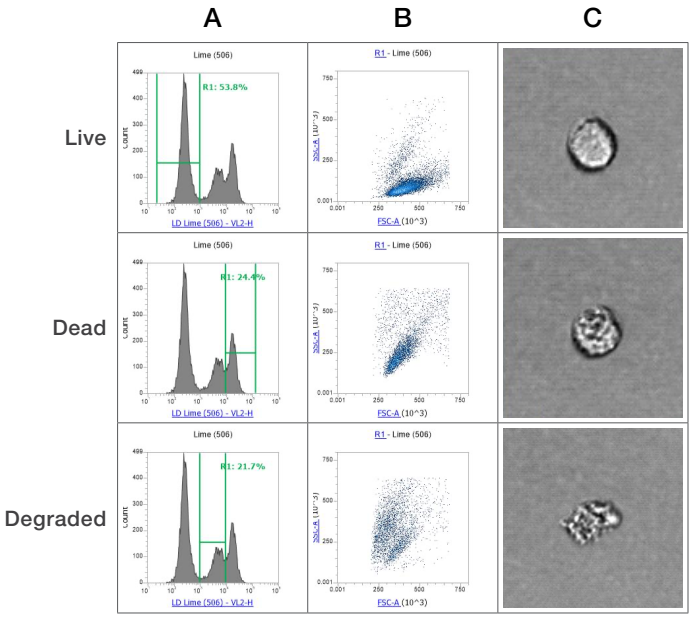


Figure 7. Gating strategy using LIVE/DEAD stains. (A) Histograms showing fluorescence from Jurkat cells stained with LIVE/DEAD Lime 506 stain. **(B)** Gating each peak reveals live, dead, and degraded populations. **(C)** Representative brightfield images showing morphological features that are consistent with the data in B. The images and flow cytometry data were collected simultaneously on the Attune CytPix Flow Cytometer.

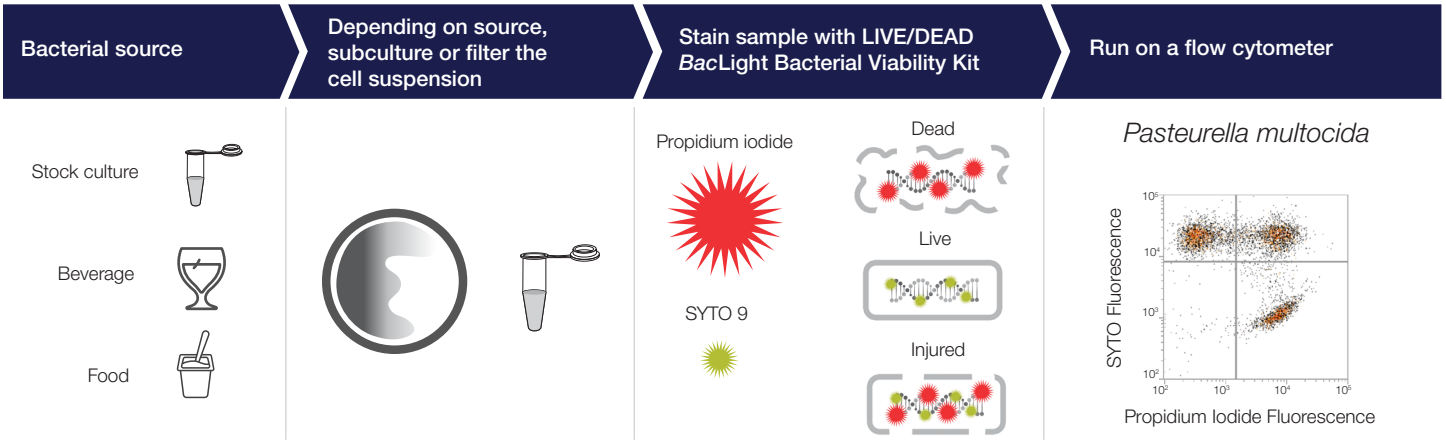


Figure 8. *Pasteurella multocida* bacteria labeled with LIVE/DEAD *Bac*Light kit stains for 15 min. Sample was analyzed on an Attune flow cytometer.

Find out more about cell viability dyes at **thermofisher.com/flow-cellviability**

Cell proliferation

Cell proliferation analysis is important for drug development and cell tracing applications. Proliferation measurements are typically made based on average DNA content or on cellular metabolism parameters. Assays can report either total live-cell numbers or measure DNA synthesis in single cells. We offer dyes, kits, and antibodies to track proliferation. Use our guide to find suitable reagents for flow cytometry assays or multicolor panels.

Example: generational tracing with CellTrace reagent

Invitrogen™ CellTrace™ reagents track cell division by analyzing cell subsets for dye dilution in successive generations (Figure 9). When cells proliferate, the fluorescence of each proliferating generation is half as bright compared with the previous generation. The CellTrace reagents help to monitor and visualize distinct generations of proliferating cells. With these reagents, you can observe one uniformly labeled cell population for each generation.

Table 5. Flow cytometry reagent selection guide for cell proliferation assays.

Product	Target	Fixable	Live-cell analysis	Application
Click-iT Plus EdU Flow Cytometry Assay Kits	Incorporation into newly synthesized DNA	Yes	Yes	Cell proliferation
BrdU	Incorporation into newly synthesized DNA	Yes	Yes	Cell proliferation
CellTrace Cell Proliferation Kits	Lysine-containing proteins	Yes	Yes	Generational analysis
Ki-67 antibody	Nuclear protein expressed in proliferating cells	Yes	Yes	Cell proliferation and cell cycle
Minichromosome maintenance (MCM2) antibody	Nuclear protein expressed in proliferating cells	Yes	No	Cell proliferation and cell cycle
Proliferating cell nuclear antigen (PCNA) antibody	Nuclear protein expressed in proliferating cells	Yes	No	Cell proliferation and cell cycle

"CellTrace Violet is the best reagent for tracking proliferation in any amenable cell type by fluorescent dye dilution and flow cytometry. Compared to CFSE, which is cytotoxic to cells when used at higher concentrations, CellTrace Violet labels cells brightly, with low toxicity and is faithfully distributed to daughter cells, ensuring the best possible peak resolution."

– Andrew Filby, Flow Cytometry Core Facility Manager and ISAC SRL Emerging Leader, Newcastle University

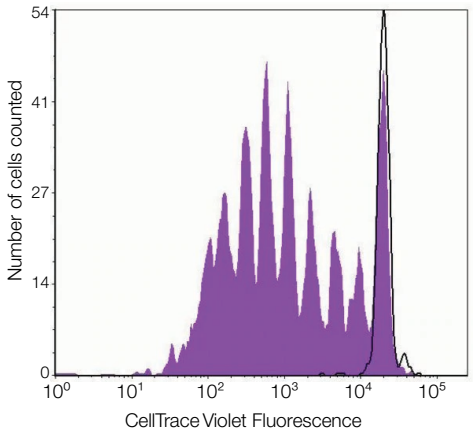


Figure 9. Tracing cell divisions with CellTrace reagent. Human peripheral blood lymphocytes were harvested and stained using the Invitrogen™ CellTrace™ Violet Cell Proliferation Kit. The violet peaks represent successive generations of cells stimulated with Invitrogen™ mouse anti–human CD3 and interleukin-2, and grown in culture for 7 days. The peak outlined in black represents cells that were grown in culture for 7 days with no stimulus.

Find out more about cell proliferation reagents at **thermofisher.com/flow-cellproliferation**

Flow cytometry beads

Beads for unmixing and compensation

Emission profiles of fluorophores are broad, which can result in overlapping profiles that require unmixing and compensation for signal correction. Unmixing and compensation can be performed using beads, particularly when cell samples are limited or when a positive population is needed.

UltraComp eBeads Spectral Unmixing Beads for unmixing and compensation

Invitrogen™ UltraComp eBeads™ Spectral Unmixing Beads are designed for spectral and conventional flow cytometry, offering flow cytometry controls. These specialized beads bind to fluorophore-conjugated antibodies, delivering precise single-color spectral unmixing controls. They are compatible with mouse, rat, hamster, rabbit, and human (including recombinant) antibodies, offering broad versatility (Figure 10).

Advantages of these spectral unmixing and compensation beads include:

- Unmixing and compensation performance comparable to real cells
- Reduced fluorescence background noise for enhanced signal detection
- Bright and consistent fluorophore signals
- Compatibility with more species
- Improved unmixing accuracy yielding more reliable results; UltraComp eBeads Spectral Unmixing Beads are suitable for use with fluorophores excited by ultraviolet (355 nm), violet (405 nm), blue (488 nm), green (532 nm), yellow-green (561 nm), red (633–640 nm), and IR (785 nm) lasers.

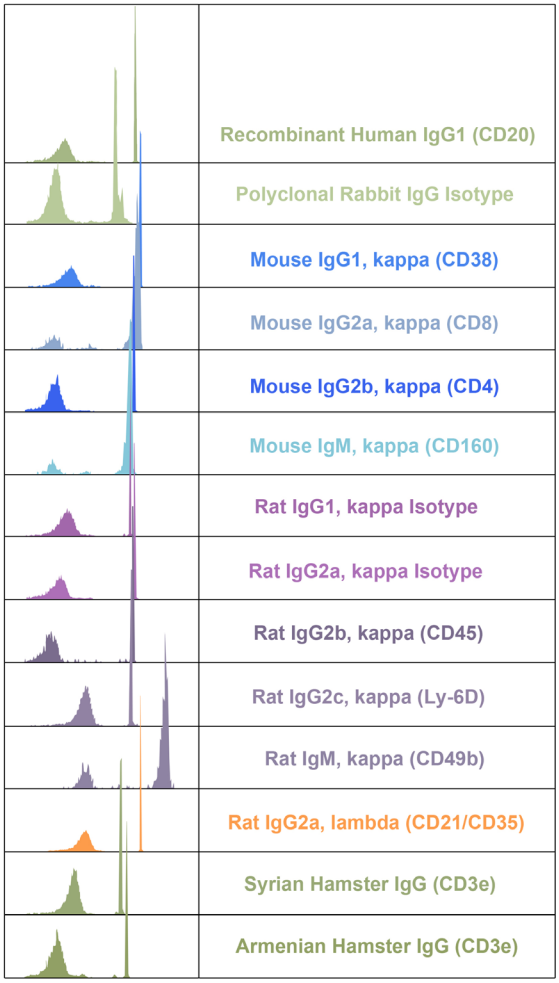


Figure 10. Staining of UltraComp eBeads Spectral Unmixing Beads with 13 different antibody species. Beads were stained with 0.25 µg of each antibody and analyzed by flow cytometry.

Table 6. Invitrogen™ antibody compensation beads.

	UltraComp eBeads™ Spectral Unmixing Beads	UltraComp eBeads™ Plus Compensation Beads	UltraComp eBeads™ Compensation Beads	AbC™ Total Antibody Compensation Bead Kit
Spectral flow cytometry	+++	+	Not recommended	Not recommended
Conventional flow cytometry	+++	+++	+	+
Cell sorting	Not recommended	Not recommended	Not recommended	Yes
Scatter properties of beads are similar to lymphocytes	Yes	Yes	Yes	No
Species reactivity*	Hu, Ms, Rb, Rt, Hm	Hu, Ms, Rb, Rt, Hm	Ms, Rt, Hm	Ms, Rt, Hm, Rb
Laser compatibility	UV to IR	UV to 633 nm	405 to 633 nm	488 to 633 nm
Single vial product format	Yes	Yes	Yes	No
Cat. No.	U20250	01-3333-42	01-2222-42	A10497

* Hm=hamster; Hu=human; Ms=mouse; Rb=rabbit; Rt=rat.

Find out more about unmixing and compensation beads at **thermofisher.com/compbeads**

Counting beads

Absolute cell counts is a method for quantifying cell concentration or absolute count of cells in a sample. Benefits of our absolute counting beads include:

- A wide range of fluorophores to fit a broad spectrum (Figure 11)
- Accommodate most cell sizes with increased percentage of singlets

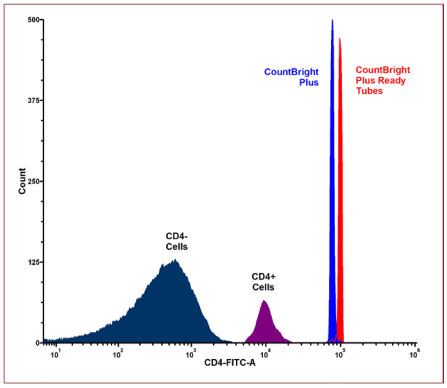


Figure 11. Invitrogen™ CountBright™ Plus Absolute Counting Beads and Invitrogen™ CountBright™ Plus Ready Tubes, spanning UV-NIR emission, show broader range of fluorophores. CountBright Plus Beads (blue) and CountBright Plus Ready Tubes (red) can be detected simultaneously with CD4-FITC–stained cells in lysed whole blood when excited with a blue laser (488 nm) with a 520/20 emission filter.

Table 7. Cell counting beads selection guide.

	CountBright Plus Ready Tubes	CountBright Plus Absolute Counting Beads	CountBright Absolute Counting Beads
Sample type	Any type, including whole blood and lysed/no-wash whole blood		
Bead size	4 µM	4 µM	7 µM
Excitation (nm)	UV to 808	UV to 808	UV to 635
Emission (nm)	385 to 860	385 to 860	385 to 800
Parameter measured	Number of cells	Number of cells	Number of cells
Cat. No.	C40000	C36995	C36950
Sample format	Lyophilized	Liquid	Liquid

- The original Invitrogen™ CountBright™ Absolute Counting Beads are still available, but not compatible with IR-excitable fluorophores.
- Countbright Plus Ready Tubes enable all the advantages of CountBright Plus Absolute Counting Beads in a convenient ready-to-use format that improves the accuracy and repeatability of cell counting, and facilitates convenient room temperature storage for up to 12 months. Each tube is supplied with a precalibrated quantity of CountBright Plus beads, removing errors associated with the handling and pipetting of beads.

Calibration and size beads

Instrument calibration is critical to collecting and analyzing accurate experimental data. Our beads are designed to help ensure robust flow cytometer performance.

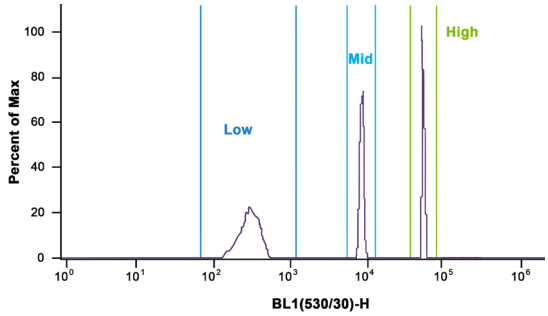


Figure 12. ERF particles provide three fluorescence intensities.

Table 8. Invitrogen™ calibration beads.

	Size calibration		Instrument control	Alignment control	Fluorescence standardization
Product	Flow Cytometry Size Calibration Kit	Flow Cytometry Sub-micron Particle Size Reference Kit	Rainbow Calibration Particles	Alignflow™ Flow Cytometry Alignment Beads	AccuCheck™ ERF and ViroCheck ERF Reference Particles
Use	Size reference	Size reference	Routine calibration of flow cytometers	Calibrate laser alignment	Standardization and calibration for inter- and intra-instrument data comparisons
Emission	No fluorescence	Green fluorescence	400–680 nm	3 types: 400–470 nm (for UV lasers), 515–660 nm (for blue lasers), or 645–680 nm (for red lasers)	AccuCheck ERF Beads, 415–910 nm ViroCheck ERF Beads, 390–910 nm
Bead size	6 sizes: 1.0–15 µm range	6 sizes: 0.02–2.0 µm	3.0–3.4 µm	2 sizes: 2.5 or 6.0 µm diameter	AccuCheck ERF Beads, 3.2 µm ViroCheck ERF Beads, 100 nm, 200 nm, 500 nm
Cat. No.	F13838	F13839	A34305	2.5 µm: A16502, A16500, A16501 6.0 µm: A16505, A16503, A16504	AccuCheck ERF Beads, A55950 ViroCheck ERF Beads, V10425

Find out more about flow cytometry beads and controls at thermofisher.com/flow-controls

Running experiments: Attune flow cytometers, a family built for accelerated discovery

Seamlessly integrating high-speed, acoustic focusing technology for every lab's needs

Run samples faster and achieve greater resolution—with minimal concern about sample loss due to clogging. Pairing an Attune flow cytometer with an Invitrogen™ CytKick™ Autosampler or an Invitrogen™ CytKick™ Max Autosampler combines precision and performance. The Attune flow cytometer family is engineered to set a new standard in flow cytometry, featuring exceptional acoustic focusing technology that uses sound waves to align cells precisely within the flow cell. This innovative approach surpasses traditional methods, resulting in faster processing speeds, exceptional data quality, and increased throughput.

- **Acoustic focusing technology**—a core feature across all Attune instruments, enabling speed, reliability, and clog-resistant operation for enhanced productivity and uptime
- **Reliability and precision**—trusted by researchers worldwide for delivering accurate and reproducible results

- **Ease of use**—designed with user-friendly interfaces and automated features to simplify lab workflows
- **Comprehensive support**—backed by exceptional customer service and technical support to help ensure your success
- **End-to-end solutions**—from instruments to reagents and automation, Thermo Fisher Scientific offers a comprehensive suite of products to meet your flow cytometry needs

Table 9. Choosing the right Attune flow cytometer model.



	Attune Xenith Flow Cytometer	Attune CytPix Flow Cytometer	Attune NxT Flow Cytometer
Description	Advanced spectral capabilities including UV and NIR lasers, supporting both traditional compensation and spectral unmixing analysis.	A high-resolution brightfield camera and automated image analysis software for morphometric analysis in combination with standard fluorescence and scatter parameters.	Advanced, high throughput, acoustic focusing, benchtop flow cytometer with up to 14 color/16 parameter flow cytometry analysis.
Number of lasers	6 (fixed)	2 to 4	1 to 4 (upgradeable)
Optics (fluorescence detection)	57 channels with wavelength-tuned photomultiplier tubes (PMTs) (51 fluorescence channels); user-changeable, keyed filters	2 scatter channels, up to 14 color channels with wavelength-tuned PMTs; user-changeable, keyed filters	

Attune Xenith Flow Cytometer

The spectrally enabled Attune Xenith Flow Cytometer delivers reliable, clog-resistant performance with acoustic-assisted hydrodynamic focusing and automated maintenance—so you can focus on results, not downtime. Equipped with UV and NIR lasers, it supports both traditional compensation and spectral unmixing for flexible, high-quality analysis. Built for high-throughput workflows, the Attune Xenith Flow Cytometer handles challenging samples with ease and consistency.

Attune CytPix Flow Cytometer

With the Invitrogen™ Attune™ CytPix™ Flow Cytometer, you can easily and rapidly highlight structural features of large populations. Using the automated image analysis software, you can rapidly analyze images using trained image processing models to generate morphology parameters that enhance your gating strategy by including cells of interest while excluding aggregates, unwanted cells, and debris. The morphology parameters can also help you gain new insights into sample biology, such as cell-cell interactions.

Find out more about the Invitrogen™ Attune™ NxT Flow Cytometer, and other Invitrogen™ instruments and robotics at thermofisher.com/attune

Cell sorting and analysis: Bigfoot cell sorter

The Invitrogen™ Bigfoot™ Spectral Cell Sorter integrates advanced fluorescence-activated cell sorting technology that enables simple operation and a wide range of cell sorting applications. It combines performance, safety, and ease-of-use to fit seamlessly into the workflows of both individual labs and core facilities. The Bigfoot instrument is capable of spectral unmixing or conventional compensation (Table 11) for high-parameter, high-throughput cell sorting experiments and maximum flexibility.

- **Fast**—sort rates >70,000 events per second (EPS) and analysis rates of >100,000 EPS
- **Flexible**—capable of six-way sorting into tubes, four-way sorting into 96-well plates, eight-way sorting into 384-well plates, or straight-down sorting into 1,536-well plates, and multiple input options with temperature control, giving flexibility for all sorting applications
- **Precise**—custom-designed, programmable-logic hardware with algorithms developed specifically for the challenges presented by sorting; the resulting architecture allows operators to use either compensation or spectral unmixing in real time at a sort rate of >70,000 EPS
- **Safe**—an adjustable sash maintains containment while allowing easy access to the nozzle and sample/sort areas, enabling constant user protection. A separate dedicated aerosol management system, HEPA filter, and software provide containment warnings and increase flow if a clog is detected.
- **Easy to use**—automated software helps provide quick start-up, automated calibration, and accurate quality control (QC) combined with an experiment designer, intuitive interface, and efficient shutdown; remote access capability allows you to start up your instrument before reaching your lab, and system health information and email notifications save time and streamline your workflow

Determination of post-sort purity requires a flow cytometric analysis of the collected sample, which was performed on the Bigfoot instrument (Figure 13). The Bigfoot Spectral Cell Sorter can resolve high-dimensional data by unmixing the spectral signatures of overlapping dyes.

This allows greater panel expansion and consequently increases the amount of information that can be gathered from each sample. We have demonstrated that this 28-color panel can be used to identify up to 20 different populations from one sample. From those populations, the Bigfoot instrument can sort six ways simultaneously, with high efficiency and purity, including several rare subsets from three tissue types.

For the complete data set, visit thermofisher.com/bigfootdata

See full technical specifications

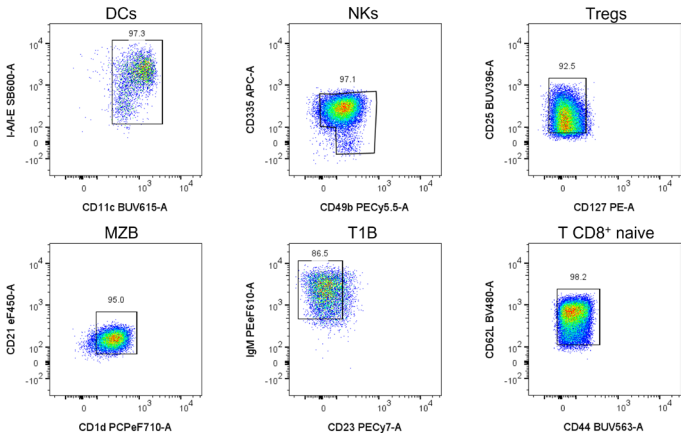


Figure 13. Purity check of sorted spleen cells. After sorting and assessing total sort numbers, the samples were rerun on the Bigfoot instrument for purity assessment. The gates were not adjusted for the post-sort purity assessment.

Find out more about the Bigfoot Spectral Cell Sorter at **thermofisher.com/bigfoot**

Table 10. Specifications for a Bigfoot Spectral Cell Sorter.

Excitation lasers (nm)	349, 405, 445, 488, 532, 561, 594, 640, and 785
Optical power	Free space delivery of 349 nm (100 mW), 405 nm (100 mW), 445 nm (200 mW), 488 nm (125 mW), 532 nm (100 mW), 561 nm (100 mW), 594 nm (100 mW), 640 nm (100 mW), and 785 nm (100 mW)
Beam alignment	Fixed, 7 spatially separated pinholes
Detection parameters	55 fluorescence + 5 scatter
Scatter parameters	Standard FSC and SSC, 488 nm; small particle FSC, 405 nm; depolarized FSC and SSC, 488 nm
Scatter resolution	<0.2 µm scatter resolution from background with small particle detector
Pulse measurement	Simultaneously measures peak, area, and width for sample input and output of every channel
Fluorescence sensitivity	<100 MESF for FITC, PE, and APC

Bigfoot cell sorter

"The instrument itself is a pleasure to work with, and it's fun to watch people new to flow in my lab take to it quickly and naturally."

Pratip Chattopadhyay, CEO
Talon Biomarkers

"The machine is designed flexibly for a core facility. In fact, I call it the Swiss army knife of cytometers…."

Rachael Walker, Head of Flow Cytometry Babraham Institute, Babraham, Cambridgeshire, England

See full Bigfoot customer stories

Attune Xenith Flow Cytometer

"The Attune Xenith instrument is a fast, clog-resistant spectral cytometer that's easy to use for existing Attune NxT cytometer or spectral users. Its low-maintenance design and reliable performance make it a strong addition to any busy flow core."

Kathryn Fox, PhD, SCYM(ASCP)CM—Flow Lab Technical Manager and Dagna Sheerar, SCYM(ASCP)CM - Flow Cytometry Director
University of Wisconsin Carbone Cancer Center Flow Cytometry Laboratory

See full Attune customer stories

Services and support

Instrument service plans and warranties

Extended-coverage service plans are available at the time of instrument purchase. These service plans can help you can maximize system uptime, reduce overall repair costs, get rapid repair by a manufacturer-trained and certified field service engineer (FSE), extend instrument life, and help keep your instrument running at peak performance. Choose from a variety of service options that balance budget, productivity, uptime, and regulatory requirements. Plans start with the most basic repair models and scale to premium offerings, including advanced support and compliance services.

Build a personalized service quote at **thermofisher.com/serviceselector**

"Our team includes a variety of experienced professionals with an average of 14 years of research experience. While we are technically oriented, our focus is the achievement and satisfaction of our customers and that is how we measure our own success."

– Ricky Williams, Commercial Global Service and Support

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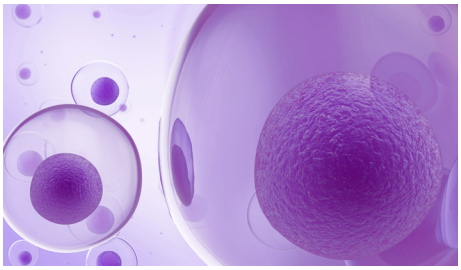

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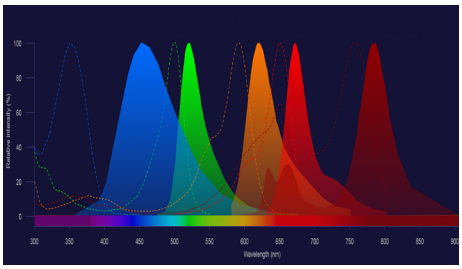

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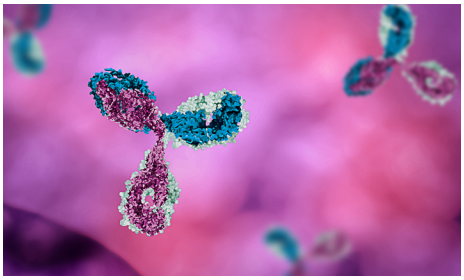
Flow cytometry antibodies



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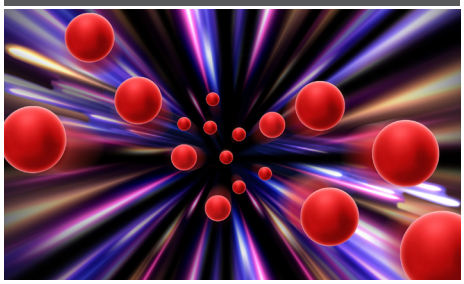
Unmixing and compensation bead controls



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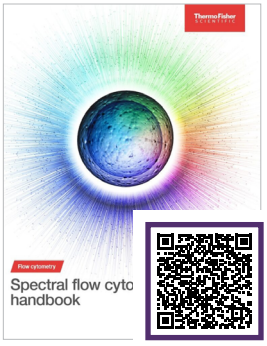
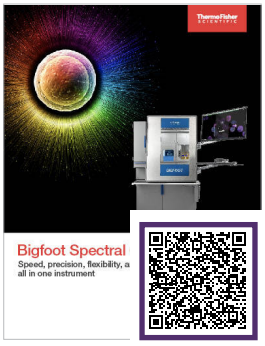


Spectral flow and protocols handbook

Attune flow cytometer family guide

Attune Xenith Flow Cytometer brochure



Bigfoot Spectral Cell Sorter brochure

Flow cytometry in microbiology handbook

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