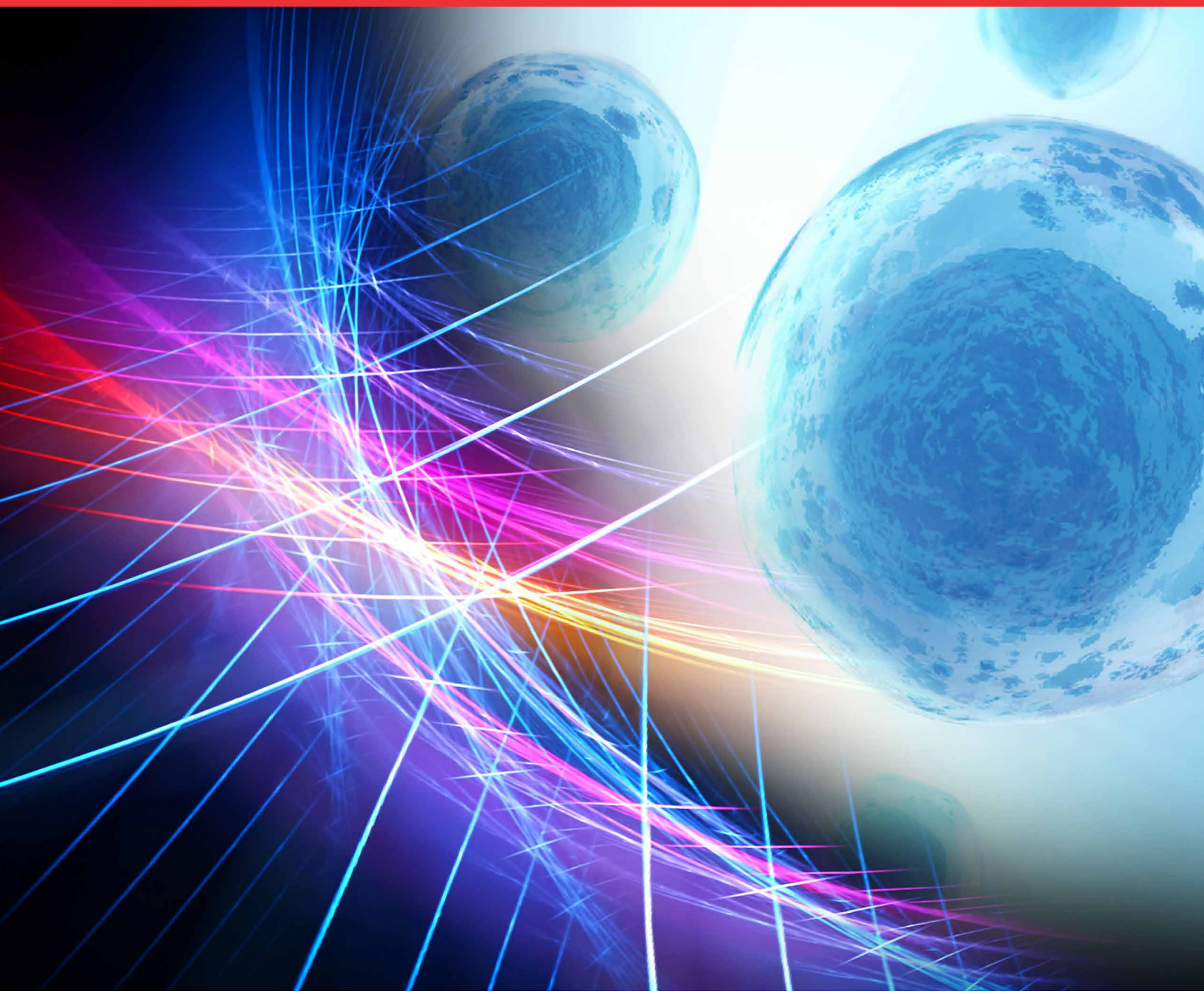
invitrogen



Flow cytometry capabilities guide

Sample preparation | Fluorophore selection | Flow cytometry antibodies and assays | Attune NxT Flow Cytometer | PrimeFlow RNA Assay | Fluorophore and reagent poster

ThermoFisher SCIENTIFIC

Getting started

Flow cytometry enables 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 in order 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 the Invitrogen™ Attune™ NxT Flow Cytometer, in the following areas:

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

Flow cytometry workflow—what you will need

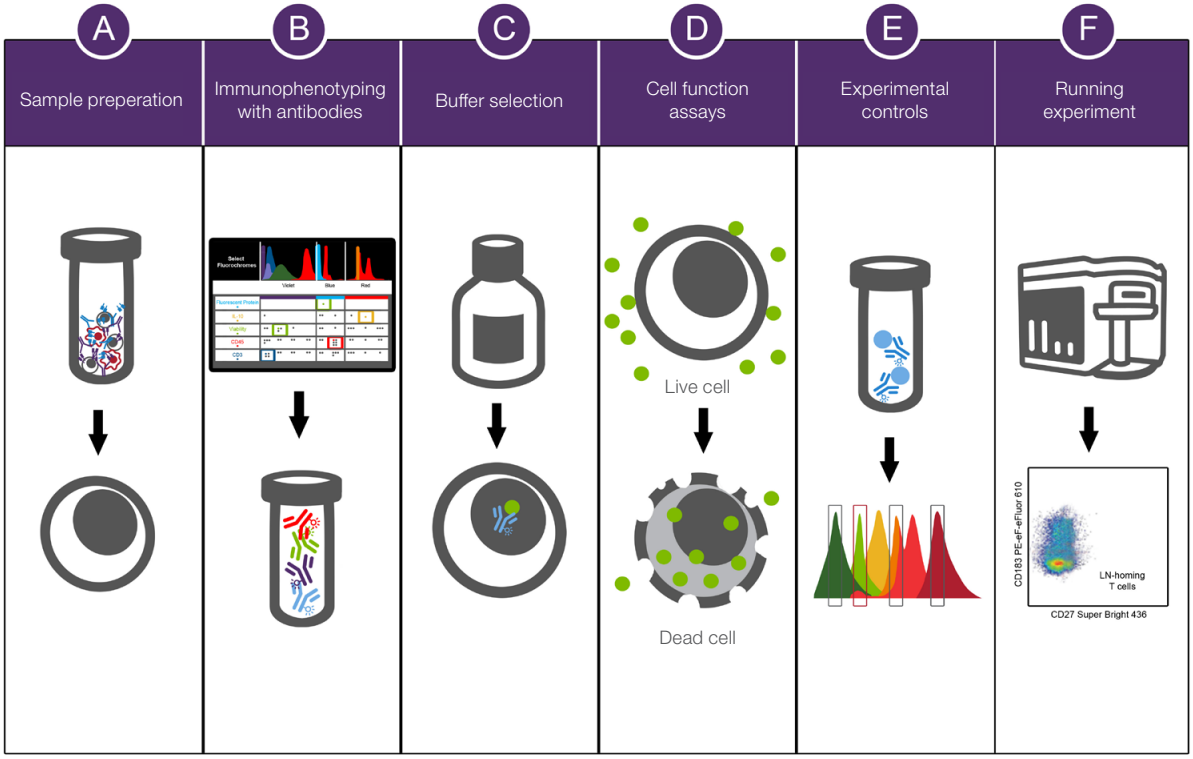


Figure 1. Flow cytometry workflow. Planning your workflow in advance as outlined will help generate a successful experiment.

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 extremely low endotoxin levels to use in cell culture
- The Invitrogen™ eBioscience™ Cell Stimulation Cocktail at a ready-to-use concentration

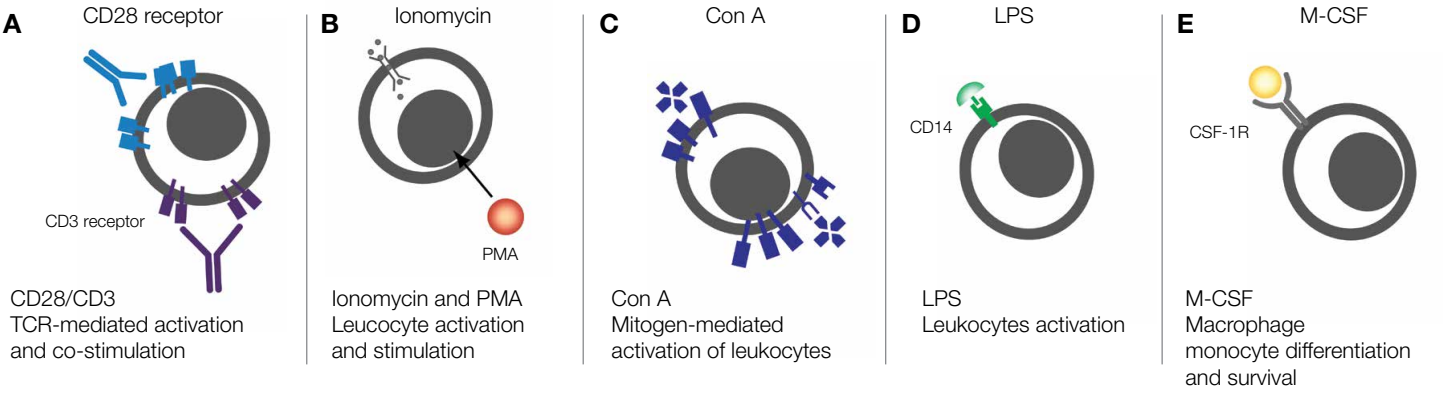


Figure 2. Cell stimulation reagents. (A) Functional-grade antibodies (e.g., anti-CD3 and anti-CD28) or Invitrogen™ Dynabeads™ magnetic beads for T cell activation and expansion. **(B)** Invitrogen™ eBioscience™ Cell Stimulation Cocktail comprising phorbol 12-myristate 13-acetate (PMA), a protein kinase activator, and ionomycin, a calcium ionophore, stimulate 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.

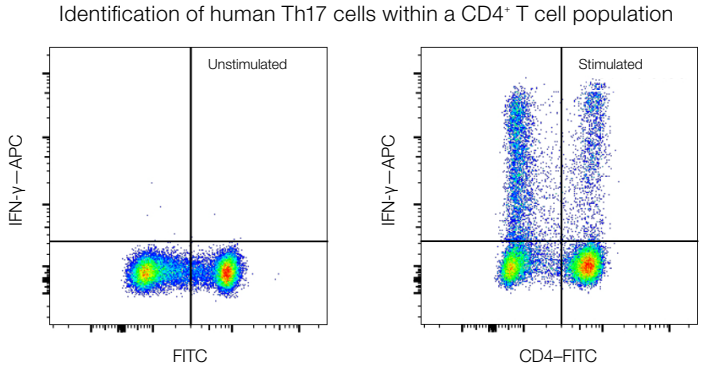


Figure 3. Identification of human Th17 cells within a CD4⁺ T cell population. Normal human peripheral blood cells were unstimulated (left) or stimulated with eBioscience Cell Stimulation Cocktail plus protein transport inhibitors (500X) (right). Cells were fixed and stained intracellularly with Invitrogen™ anti–human CD4 APC and anti–human IFN-γ conjugated to eBioscience™ PE-eFluor™ 610 dye, 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/flow-assays

Immunophenotyping with 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

Select from one of the largest portfolios of primary conjugated antibodies specifically developed for flow cytometry applications. 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.

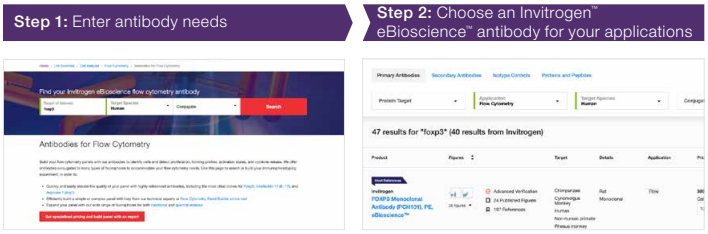


Figure 4. Antibody search tool to find information and purchase antibodies. (Left) Antibody application data from customer publications and internal testing data. (Right) A list of antibodies can be purchased, or saved and shared for later use.

* 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.

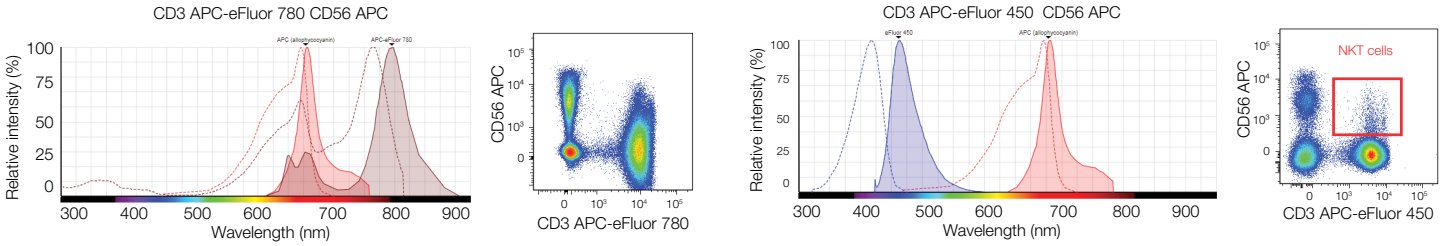


Figure 5. Normal human peripheral blood cells were stained with anti–human CD3 antibody conjugated with Invitrogen™ eBioscience™ APC-eFluor™ 780 dye (left) or eFluor™ 450 dye (right), as well as anti–human CD56 antibody conjugated with APC dye. Cells in the lymphocyte gate were used for analysis.

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
Organic dyes—small, stable molecules	Original	• FITC is cost-efficient	FITC
	Pacific dyes	• Some of the dimmest dyes	Pacific Blue
			Pacific Orange
	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
			Alexa Fluor 488
			Alexa Fluor 532
			Alexa Fluor 561
			Alexa Fluor 647
			Alexa Fluor 660
			Alexa Fluor 700
	eFluor organic dye	• Engineered for detection for flow cytometry • Named for their emission wavelength	eFluor 450
			eFluor 506
			eFluor 660
Large, protein-based molecules	Original	• Cost-efficient • Some of the brightest dyes available	APC
			PE
			PerCP
	Tandem dyes	• Dyes occupy different channels from the donor molecule, and this can be used to build larger panels	APC-Cyanine5
			APC-Cyanine7
			PE-Cyanine5 (TRI-COLOR)
			PE-Cyanine5.5
			PE-Cyanine7
			PE–Texas Red
			PerCP-Cyanine5.5
			PE–Alexa Fluor 610
			PE–Alexa Fluor 700
			APC–Alexa Fluor 750
			PE–eFluor 610
			PerCP–eFluor 710
			APC–eFluor 780
Polymer dyes—recent dye innovation	Super Bright dyes and their tandems	• Excited by the 405 nm violet laser • Minimal spillover into other channels • Add Super Bright Complete Staining Buffer (Cat. No. SB-4401-42) when using two or more polymer dyes to lower background levels	Super Bright 436
			Super Bright 600
			Super Bright 645
			Super Bright 702
			Super Bright 780

Creating a flow cytometry panel

The Invitrogen™ Flow Cytometry Panel Builder is a free online tool to help select antibody conjugates and reagents for a multicolor flow cytometry panel (Figure 6). 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 experiment or add antibodies and reagents to an existing panel
- Check fluorophore emission spectra with the built-in SpectraViewer
- Export an Excel™ document with your antibody choices, or order directly

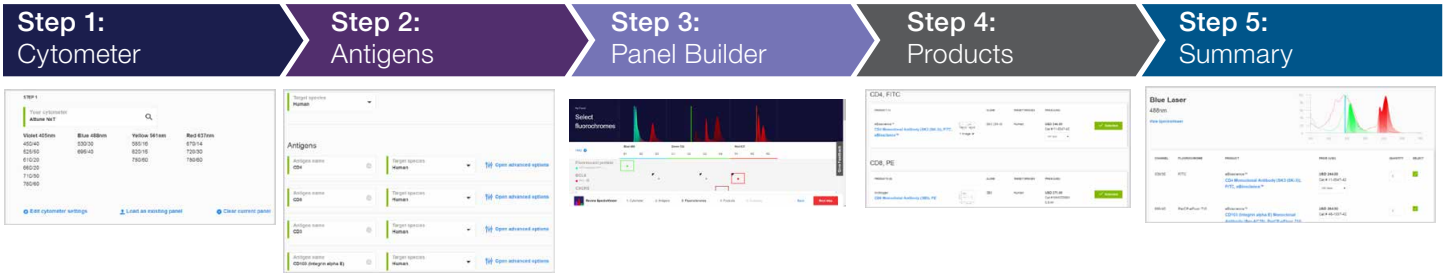
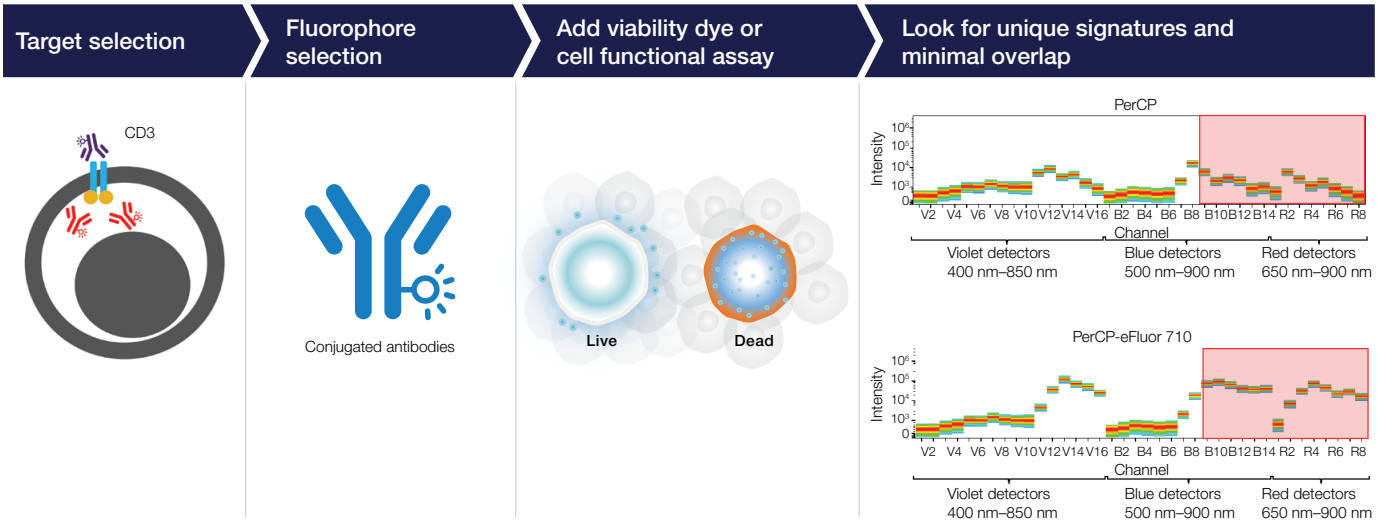


Figure 6. The Flow Cytometry Panel Builder simplifies experimental design with a 5-step strategy.

Plan your experiment at **thermofisher.com/flowpanel**

Application spotlight—immunophenotyping on a spectral flow cytometer

- Standard panel design rules apply
- Invitrogen™ fluorescent probes and reagents are suitable for all cytometry instrumentation, including spectral flow cytometers; use fluorophores designed for use with spectral instruments including Alexa Fluor 561 and Alexa Fluor 660 dyes
- Many previously incompatible labeling dyes and functional reagents, including PerCP and PerCP eFluor 710 dyes, can now be used together in your expanded multicolor application
- Expand your panel with Alexa Fluor 532, Pacific Orange, eFluor 450, and Super Bright 436 labels



	Violet laser (405 nm)								Blue laser (488 nm)								Red laser (635 nm)			
	Super Bright 436	eFluor 450	eFluor 506	Pacific Orange	Super Bright 600	Super Bright 645	Super Bright 702	Brilliant Violet 785	FITC	Alexa Fluor 532	PE	PE-eFluor 610	PE-Cy5	PE-Cy5.5	PerCP-eFluor 710	PE-Cy7	APC	Alexa Fluor 647	Alexa Fluor 700	Alexa Fluor 780
Super Bright 436																				
eFluor 450																				
eFluor 506																				
Pacific Orange																				
Super Bright 600																				
Super Bright 645																				
Super Bright 702																				
Brilliant Violet 785																				
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PE																				
PE-eFluor 610																				
PE-Cy5																				
PE-Cy5.5																				
PerCP-eFluor 710																				
PE-Cy7																				
APC																				
Alexa Fluor 647																				
Alexa Fluor 700																				
APC-eFluor 780																				

Table 2. Staining spread matrix of 20 Invitrogen fluorophores that can be used simultaneously on a three-laser spectral flow cytometer.* All fluorophores were compared using anti-CD4 antibody conjugates to demonstrate the level of spread among dyes. The fluorophore in each row impacts the resolution of the fluorophore in each column. Although all dyes in the matrix can be used together, the darker red shading means one fluorophore has increased spread into the other and needs closer attention during panel design and data interpretation.

* All spectral flow cytometry data shown were generated by Cytek Biosciences on a Cytek™ Aurora™ spectral flow cytometer three-laser system and analyzed using SpectroFlo™ software.

Find out more about panel design at **thermofisher.com/spectralflowcytometry**

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 3. 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 4. 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 (RUO and clinical research–grade) or **Intracellular Fixation and Permeabilization Buffer Set (RUO)**	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
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/flow-sample**

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 7).

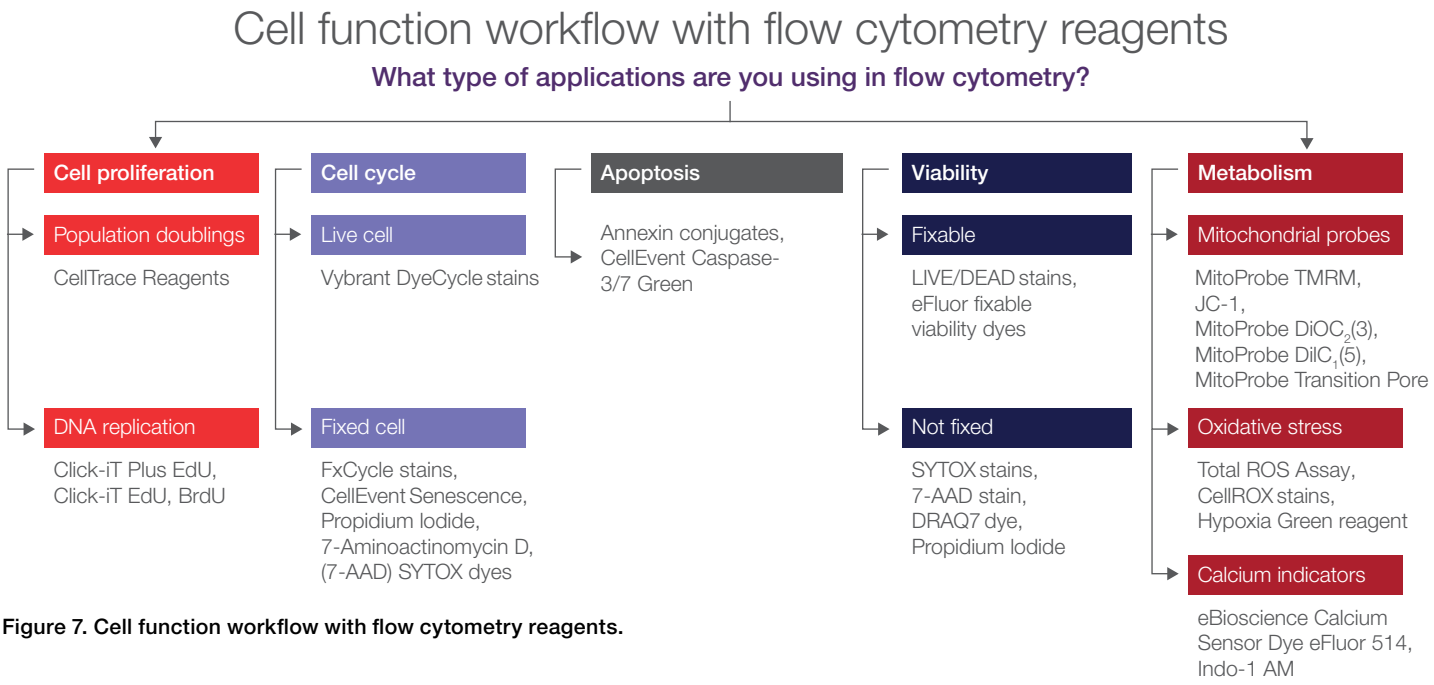


Figure 7. Cell function workflow with flow cytometry reagents.

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 5. Cell viability dyes selection guide.

Laser	Live cell/nonfixable stains	Fixable stains
UV	SYTOX Blue (450/50*)	LIVE/DEAD fixable blue dead cell stain (350/40*)
405 nm	SYTOX Blue (450/50*)	LIVE/DEAD fixable violet dead cell stain (450/40*)
		LIVE/DEAD fixable aqua dead cell stain (530/50*)
		LIVE/DEAD fixable yellow dead cell stain (585/42*)
488 nm	SYTOX Green (530/30*)	LIVE/DEAD fixable green dead cell stain (530/30*)
	SYTOX AADvanced (>650*)	LIVE/DEAD fixable red dead cell stain (>650 or 600/20*)
	Propidium Iodide (~617*)	
532 nm	SYTOX Orange (585/42*)	LIVE/DEAD fixable red dead cell stain (>650 or red bandpass*)
	SYTOX AADvanced (>650*)	
	Propidium Iodide (~617*)	
561 nm	LDS 751 (700/20*)	LIVE/DEAD fixable red dead cell stain (>650 or red*)
633/5 nm	SYTOX Red (660/20*)	LIVE/DEAD fixable far-red dead cell stain (660/20*)
		LIVE/DEAD fixable near-IR dead cell stain (780/60*)

* Recommended filters (nm).

Find out more at **thermofisher.com/flow-assays**

Example: avoid inaccurate analysis with LIVE/DEAD assay

When choosing a viability dye to stain cells post-fixation, it is important to select one that is retained in the cell post-fixation and preserves the staining pattern. Exclusion of the dead cells from the data allows cleaner separation and identification of cell populations (Figure 8). Invitrogen™ LIVE/DEAD™ Fixable Dead Cell Stains are fixable viability dyes that help to ensure accurate assessment of cell viability in samples after fixation and/or permeabilization (Figure 9).

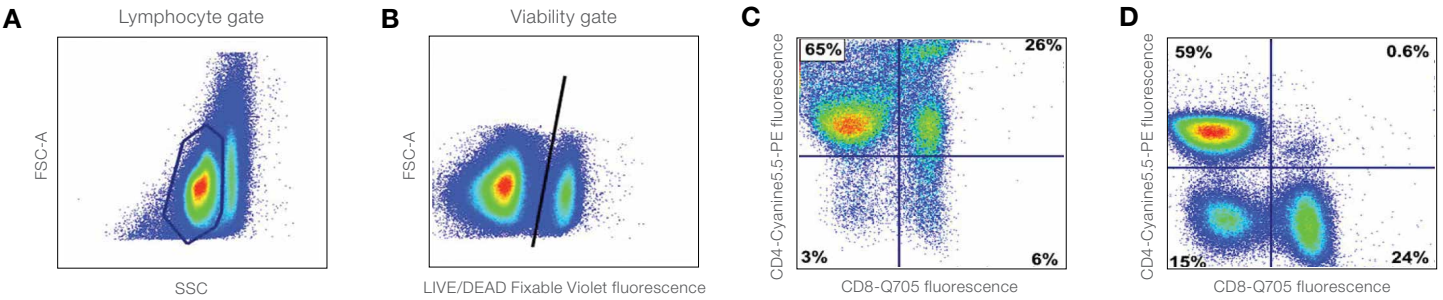


Figure 8. Exclusion of dead cells eliminates staining artifacts from analysis. After the application of a lymphocyte gate **(A)**, live and dead cells were discriminated **(B)** using the Invitrogen™ LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (Cat. No. L34963). Note the significant number of dead 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, and Lamoreaux L et al. (2006) *J Immunol Methods* 313:199–208, with permission from Elsevier.

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
- A serial dilution is not necessary—just take stained sample, dilute, and analyze
- Invitrogen™ LIVE/DEAD™ *Bac*Light™ kits can be used to quickly determine bacterial cell viability

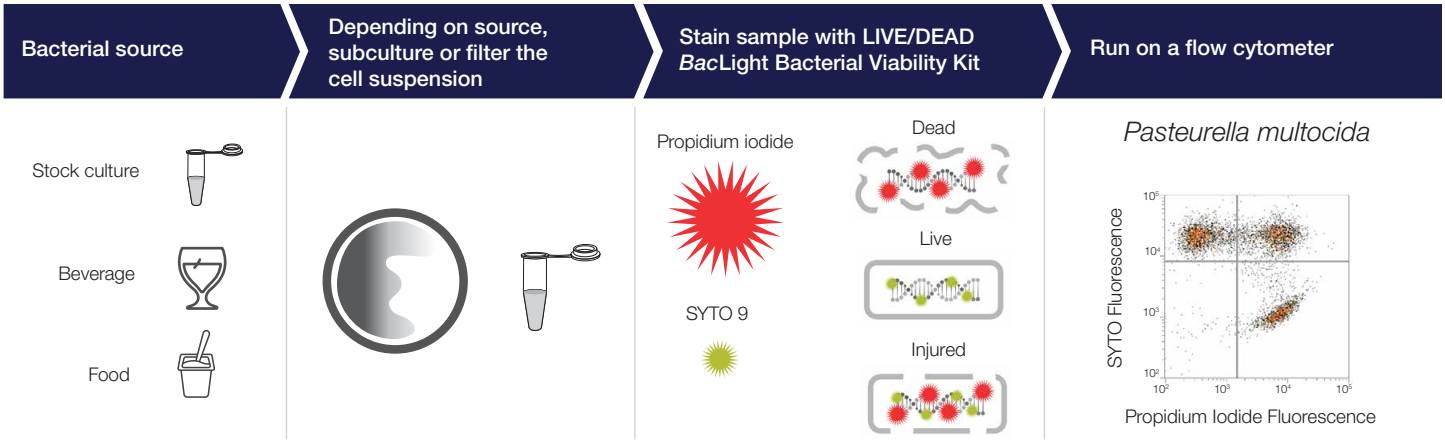


Figure 9. *Pasteurella multocida* bacteria labeled with LIVE/DEAD *Bac*Light kit stains for 15 min. Sample was analyzed on the Attune NxT Flow Cytometer.

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

Cell proliferation

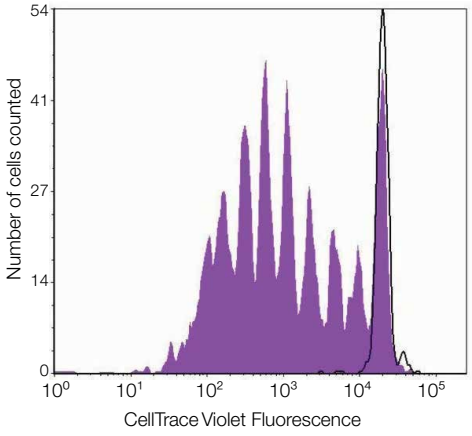
Cell proliferation analysis is important for drug development and cell tracing applications. Proliferation measurements are typically made based on average DNA content or on cellular metabolism parameters. Assays can report either total live cell numbers or measure DNA synthesis in single cells. We offer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

Table 6. 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

Example: generational tracing with CellTrace reagent

Invitrogen™ CellTrace™ reagents track cell division by analyzing cell subsets for dye dilution in successive generations (Figure 10). 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.



> "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 10. 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

RNA detection by flow cytometry

With the novel Invitrogen™ PrimeFlow™ RNA Assay, scientists can now reveal the dynamics of RNA and protein expression simultaneously within millions of single cells (Figure 11). This assay employs a proprietary fluorescence *in situ* hybridization (FISH) and branched DNA (bDNA) amplification (Figure 12) technique for simultaneous detection of up to four RNA transcripts labeled with Invitrogen™ Alexa Fluor™ 488, Alexa Fluor™ 568, Alexa Fluor™ 647, and Alexa Fluor™ 750 dyes, in a single cell using a standard flow cytometer. RNA detection may be combined with intracellular and cell-surface antibody staining to elevate the understanding of single-cell dynamics to a new dimension.

Novel product applications:

- Unmask gene expression heterogeneity at the single-cell level
- Correlate RNA and protein levels in the same cell
- Detect noncoding RNA, microRNA (miRNA), and long noncoding RNA (lncRNA)
- Evaluate viral RNA in infected cells
- Analyze mRNA expression when antibody selection is limited
- Analyze up to four RNA transcripts simultaneously
- Detect telomere DNA

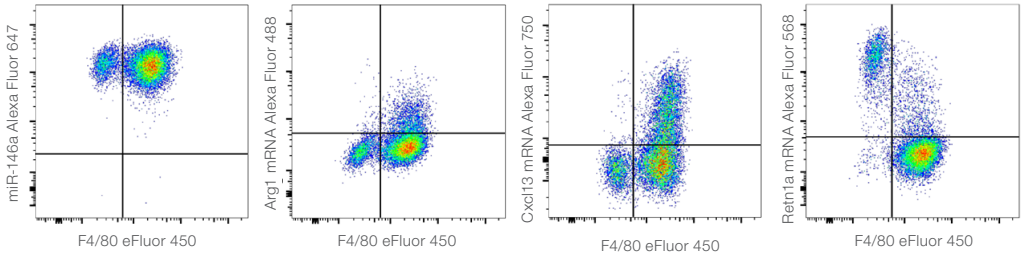


Figure 11. PrimeFlow RNA Assay detection of miR-146a, Arg1 mRNA, Cxcl13 mRNA, and Retn1a mRNA in mouse peritoneal cells. C57Bl/6 mouse resident peritoneal exudate cells were analyzed using the PrimeFlow RNA Assay. Cells were stained with Invitrogen™ eBioscience™ Anti–Mouse F4/80 eFluor 450 and Anti–Mouse CD11b PE-Cyanine7 antibodies, then fixed and permeabilized using PrimeFlow RNA Assay buffers and protocols. Cells were then hybridized to label RNA with Invitrogen™ Type 1 Human/Mouse miR146a Alexa Fluor 647, Type 4 Mouse Arg1 Alexa Fluor 488, Type 6 Mouse Cxcl13 Alexa Fluor 750, and Type 10 Mouse Retn1a Alexa Fluor 568 target probes. Viable $CD11b^+$ cells were used for analysis. Data show that both small peritoneal macrophages (SPM, $F4/80^-$) and large peritoneal macrophages (LPM, $F4/80^+$) were positive for miR-146a. SPM expressed high levels of Retn1a (Relm-alpha) mRNA, whereas LPM were positive for Cxcl13 mRNA and expressed low levels of Arg1 mRNA.

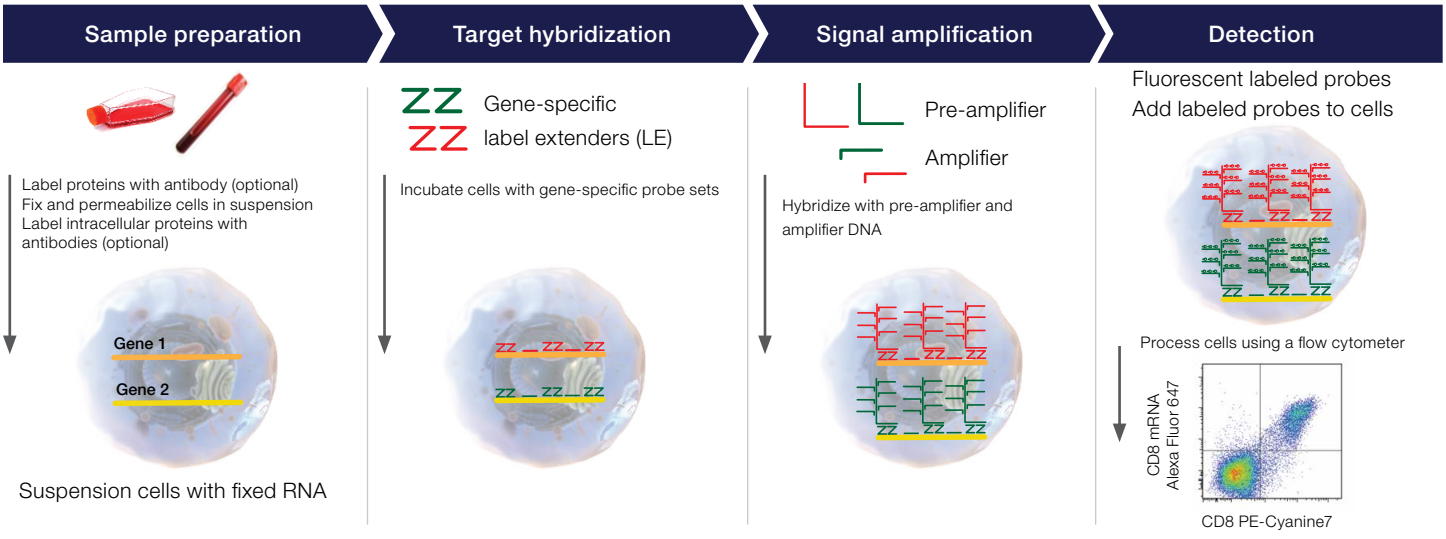


Figure 12. The PrimeFlow RNA Assay workflow. The assay workflow contains several steps: antibody staining; fixation and permeabilization, including intracellular staining, if desired; and target hybridization with a target-specific probe set containing 20–40 oligonucleotide pairs.

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

Compensation and instrument beads

Compensation beads for flow cytometry

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

The latest generation of compensation beads

Build flow cytometry panels with more accurate compensation using new Invitrogen™ UltraComp eBeads™ Plus Compensation Beads. When a fluorophore-conjugated antibody is added to the beads, both positive and negative populations result. UltraComp eBeads Plus Compensation Beads now offer:

- Increased species reactivity including rabbit- and human-origin antibodies (Figure 13)
- Compatibility with fluorophores excited by ultraviolet (355 nm), violet (405 nm), blue (488 nm), green (532 nm), yellow–green (561 nm), and red (633–640 nm) lasers
- Better compensation resolution for antibodies conjugated with Invitrogen™ eBioscience™ Super Bright 780, Brilliant Violet 711, or Brilliant Violet 786 dyes

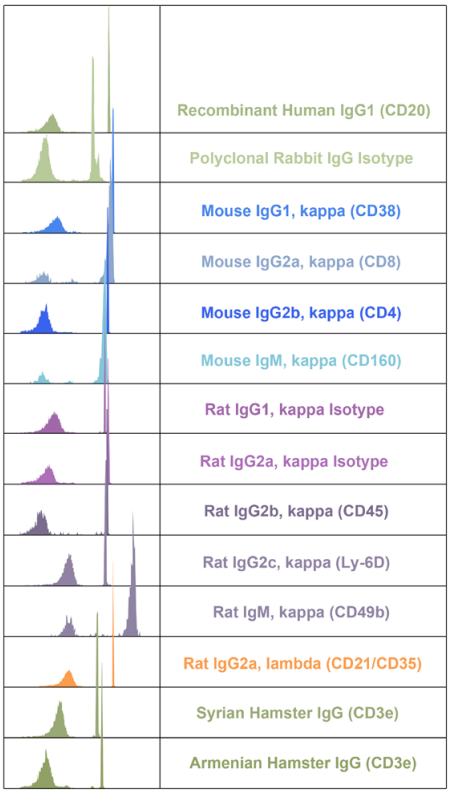


Figure 13. Staining of UltraComp eBeads Plus Compensation Beads with 14 different antibody species. Beads were stained with 0.25 µg of each antibody and analyzed by flow cytometry.

Table 7. Invitrogen™ antibody compensation beads.

	UltraComp eBeads™ Plus beads	UltraComp eBeads™ beads	OneComp eBeads™ beads	AbC™ Total Antibody Compensation Bead Kit*	ArC™ Amine Reactive Compensation Bead Kit	GFP BrightComp eBeads™ beads
Application	Immunophenotyping				Cell viability assay	GFP expression; beads are present at 3 levels of GFP-like intensity
Reactivity	Human, rabbit, hamster, mouse, and rat antibodies	Hamster, mouse, and rat antibodies with recognition of the kappa and lambda chains		Hamster, mouse, rabbit, and rat antibodies	LIVE/DEAD™ fixable dead cell stains*	GFP isoforms
Format	One vial: dispense as a single drop			1 vial positive beads, 1 vial negative beads		One vial: dispense as a single drop
Laser compatibility	Compatible with most standard lasers, UV to 633 nm; improved for polymer dye use from the violet laser	Compatible with most standard lasers, UV to 633 nm	Compatible with most standard lasers, but not with UV or violet lasers	Compatible with most standard lasers, UV to 633 nm		488 nm
Quantity	25 tests or 100 tests					25 tests
Cat. No.	01-3333-41 01-3333-42	01-2222-41 01-2222-42	01-1111-41 01-1111-42	A10513 A10497	A10628 A10346	A10514

* Also applicable to similar amine-reactive dyes.

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:

- Wide range of fluorophores to fit a broad spectrum (Figure 14)
- Accommodates most cell sizes with increased percentage of singlets

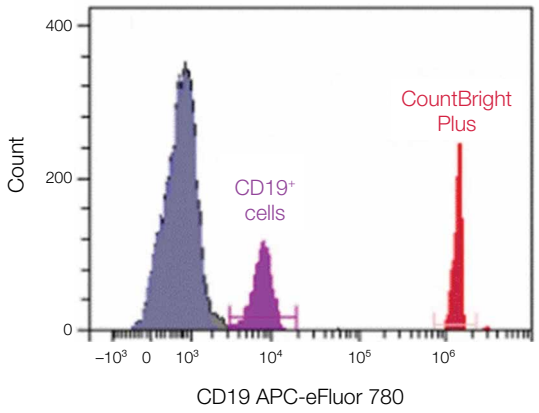


Figure 14. CountBright Plus beads can be used with a broader range of fluorophores. CountBright Plus beads (red) can be detected simultaneously with cells stained with Invitrogen™ CD19 APC-eFluor™ 780 antibody (pink) in lysed whole blood when excited with an IR laser (808 nm) with an 840/20 nm emission filter.

Table 8. Invitrogen™ absolute counting beads.

	CountBright™ Plus beads*	AccuCheck™ beads		LIVE/DEAD™ *Bac*Light™ Bacterial Viability and Counting Kit**
Parameters measured	Cell concentration in sample	• Cell concentration in sample • Pipetting accuracy		• Viability • Bacterial concentration in sample
Sample type	Any type	Whole blood		Bacteria
Bead size	4 µm	Bead A 6.40 µm	Bead B 6.36 µm	6 µm
Range	Ex: UV–800 nm Em: 385–860 nm	Bead A Ex: 488 nm Em: 575–585 nm	Bead B Ex: 635 nm Em: 660–680 nm	Ex: 488 nm Em: 617 nm, 498 nm
Cat. No.	C36995	PCB100		L34856

* The original Invitrogen™ CountBright™ Absolute Counting Beads are still available, but not compatible with IR-excitable fluorophores.

** Stains all cells, so a pure bacterial sample is required for accurate results.

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.

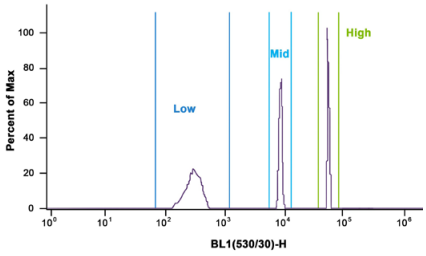


Figure 15. ERF particles provide three fluorescence intensities.

Table 9. 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 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)	415–910 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	3.2 µM
Cat. No.	F13838	F13839	A34305	2.5 µm: A16502, A16500, A16501 6.0 µm: A16505, A16503, A16504	A55950

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

Sample analysis: Attune NxT Flow Cytometer, CytKick Autosamplers, and automation

Run samples faster and achieve greater resolution—with little fear of sample loss due to clogging. The Attune NxT Flow Cytometer with Invitrogen™ CytKick™ or CytKick™ Max Autosampler combines precision with performance in a benchtop flow cytometer that is configurable with up to four lasers and 16 parameters of detection.

- **Transform your research**—get a superior level of data fidelity at speeds of up to 1 mL/min; discover rare cells and analyze more cells in a shorter period of time
- **Six fluorescence channels off the violet laser**—expand your capabilities in multicolor flow cytometry
- **Simplified sample prep**—no-wash, no-lyse sample prep options streamline your workflow
- **Flexibility**—convert between tubes and plates with a simple click of the mouse
- **Option for automation**—designed for walk-away performance with clog-resistant fluidics and robust data analysis software
- **Compatible**—mammalian cells, algae, bacteria, yeast, parasites, and plant cells successfully analyzed

Table 10. Attune NxT Flow Cytometer specifications.

Attribute	Specification
Optics	• Laser wavelength (nm): Violet 405, blue 488, green 532, yellow 561, red 637
	• Emission filters: Up to 14 color channels with wavelength-tuned photomultiplier tubes (PMTs); user-changeable, keyed filters
Fluidics	• Flow cell: Quartz cuvette gel coupled to 1.2 numerical aperture (NA) collection lens, 200 x 200 µm
	• Sample analysis volume: 20 µL–4 mL
	• Custom sample flow rates: 12.5–1,000 µL/min
	• Sample delivery: Positive-displacement syringe pump for volumetric analysis
	• Fluorescence sensitivity: ≤80 molecules of equivalent soluble fluorochrome (MESF) for FITC, ≤30 MESF for PE, ≤70 MESF for APC
	• Fluorescence resolution: CV <3% for the singlet peak of propidium iodide–stained chicken erythrocyte nuclei (CEN)
	• Data acquisition rate: Up to 35,000 events/sec, 34 parameters, based on a 10% coincidence rate per Poisson statistics
	• Maximum electronic speed: 65,000 events/sec with all parameters
Performance	• Carryover: Single-tube format: <1%
	• Forward and side scatter sensitivity: Able to discriminate platelets from noise
	• Forward and side scatter resolution: Optimized to resolve lymphocytes, monocytes, and granulocytes in lysed whole blood
	• Minimum particle size: 0.2 µm on side scatter using submicron bead calibration kit from Bangs Laboratories—0.1 µm on side scatter under following conditions: Using an Attune NxT Flow Cytometer with standard blocking configuration, an Attune NxT 488/10 Filter (Cat. No. 100083194), and Attune Focusing Fluid (Cat. No. 4488621, 4449791, or A24904) that has been passed through a 0.025 µm filter

Find out more about instruments and robotics at **thermofisher.com/attune**

Application spotlight—analyze samples for CRISPR-edited cells on the Attune NxT Flow Cytometer

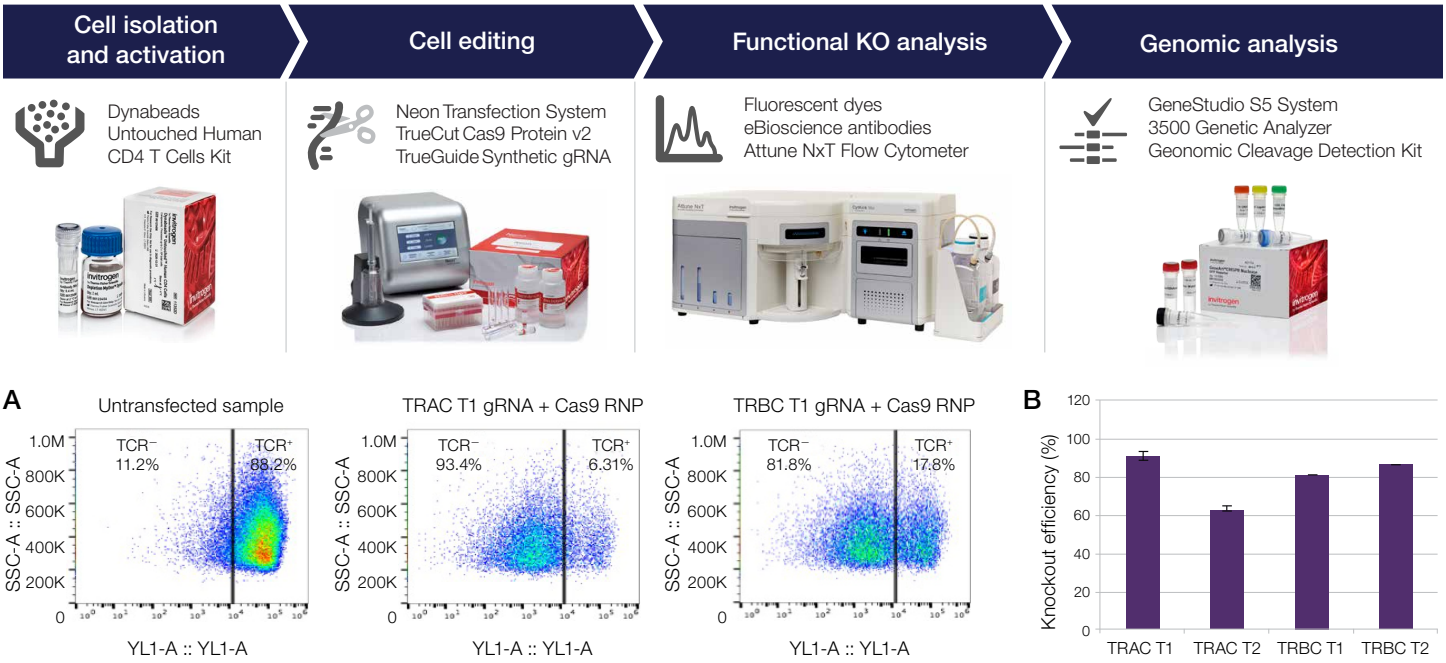


Figure 16. High-efficiency functional knockout in T cells. T cells were isolated from PBMCs (from a healthy donor) using Dynabeads magnetic beads, and then transfected with Invitrogen™ TrueCut™ Cas9 Protein v2 and Invitrogen™ TrueGuide™ Modified Synthetic sgRNAs targeting T cell receptor alpha (TRAC) or beta (TRBC) regions using the Invitrogen™ Neon™ Transfection System. **(A)** Analysis by flow cytometry following binding with antibody specific to the T cell receptor (TCR) shows >90% functional knockdown of the receptor. For both TRAC and TRBC, gRNAs specific for two different genomic DNA targets (T1 and T2) were tested, and results are shown only for the T1 target in each case. **(B)** Summary of NGS-based analysis of cleavage efficiency at two different genomic DNA targets (T1 and T2) for both TRAC and TRBC loci.

Services and support

Instrument service plans and warranties

Extended-coverage service plans are available at the time of instrument purchase. With these service plans you can maximize system uptime, reduce overall repair costs, get fast repair turnaround time from a manufacturer-trained and certified field service engineer (FSE), extend instrument life, and help keep it 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.

Technical support for help with flow cytometry experiments

Technical support and specialists assist with panel design and help choose the correct antibodies for your needs, including new experiments and quality control. Each specialist helps troubleshoot experiments and product performance issues, as well as designing and helping customers implement complex flow cytometry panels (>30 colors), all remotely via phone or email. Services are available globally.

"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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Ordering information

Product	Cat. No.
Cell stimulation reagents	
Cell Stimulation Cocktail	00-4970-93
Concanavalin A (Con A) Solution (500X)	00-4978
Lipopolysaccharide (LPS) Solution (500X)	00-4976
Anti–Human CD3, Functional-Grade Purified (clone OKT3)	16-0037
Anti–Human CD28, Functional-Grade Purified (clone CD28.2)	16-0289
Macrophage Colony-Stimulating Factor (M-CSF)	PHC9504
Flow cytometry antibodies	
eBioscience flow cytometry antibodies	**thermofisher.com/flowantibodies**
Fixatives	
eBioscience Flow Cytometry Staining Buffer	00-4222-57
FIX & PERM Cell Permeabilization Kit	GAS003
eBioscience Intracellular Fixation and Permeabilization Buffer Set	88-8824-00
eBioscience Foxp3/Transcription Buffer Set	00-5523-00
Viability dyes	
CellTrace Blue Cell Proliferation Kit, for flow cytometry	C34568
CellTrace CFSE Cell Proliferation Kit, for flow cytometry	C34570
CellTrace Far Red Cell Proliferation Kit, for flow cytometry	C34564
CellTrace Violet Cell Proliferation Kit, for flow cytometry	C34557
CellTrace Yellow Cell Proliferation Kit, for flow cytometry	C34567
LIVE/DEAD Fixable Blue Stain	L23105
LIVE/DEAD Fixable Violet Stain	L34955
LIVE/DEAD Fixable Aqua Stain	L34957
LIVE/DEAD Fixable Yellow Stain	L34959
LIVE/DEAD Fixable Green Stain	L23101
LIVE/DEAD Fixable Red Stain	L23102
LIVE/DEAD Fixable Far Red Stain	L10120
LIVE/DEAD Fixable Near-IR Stain	L10119
Bead controls	
UltraComp eBeads Compensation Beads	01-2222-41
UltraComp eBeads Plus Compensation Beads	01-3333-42
AbC Total Antibody Compensation Bead Kit	A10497
ArC Amine Reactive Compensation Bead Kit	A10346
GFP BrightComp eBeads Compensation Beads	A10514
CountBright Plus Absolute Counting Beads	C36995
AccuCheck Counting Beads	PCB100
AccuCheck ERF Reference Particles	A55950
LIVE/DEAD *Bac*Light Bacterial Viability and Counting Kit	L34856
Instruments	
Attune NxT Flow Cytometer	**thermofisher.com/attune**
CytKick Autosampler	A42901
CytKick Max Autosampler	A42973

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Find out more at **thermofisher.com/flow**

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