

invitrogen

Flow cytometry capabilities guide



Conjugated antibodies



Cell health



Flow RNA



Everyday essentials



Flow cytometry instrument

Don't settle for average—
real insight starts with single cells

ThermoFisher
SCIENTIFIC

Push the boundaries of cell analysis

Flow cytometry is an important technique central to many of the fastest-growing areas of life science research, such as immuno-oncology, antibody therapeutic development, and gene editing, because it empowers researchers to study biology at the single-cell level.

At Thermo Fisher Scientific, we are committed to accelerating your science by providing a comprehensive suite of solutions for the analysis of cells and their functions. Behind this commitment is an incredible team of scientists developing and supporting our innovative instrumentation and products, such as the Invitrogen™ Attune™ NxT Flow Cytometer, Invitrogen™ eBioscience™ flow cytometry antibodies, and Invitrogen™ flow cytometry assays and reagents.

We are focused on advancing meaningful discoveries and partnerships to make tools for cellular analysis widely accessible, affordable, and powerful for all life science scientists. When you're on your quest for significant breakthroughs, we know that you never settle for average, and neither will we.



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Flow cytometry
antibodies, assays, and reagents



Flow cytometry
instrumentation



Flow cytometry
education and support

Flow cytometry workflow capabilities

Uniquely positioned to address your workflow needs

Flow cytometry enables the simultaneous, multiparametric analysis of proteins, gene expression, and cell functions such as viability, cell cycle, apoptosis, and proliferation. This technology makes it possible to obtain a statistically relevant amount of data from a heterogeneous cell population. Whether you are identifying cell subpopulations, analyzing cells at the single-cell level, or investigating what is going on within them, flow cytometry offers significant advantages over other applications.

However, ensuring that experimental conditions are consistent and data are reproducible can be challenging in flow cytometry. In response to this dilemma, we have developed high-quality instruments and products that are validated* and optimized by our flow cytometry R&D team to help facilitate reproducible results and to reduce error, thereby saving your laboratory time and money. Additionally, using products and systems that have been designed to work together takes some of the guesswork out of flow cytometry and helps enable better, more reproducible data.

* 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.



Antibodies, assays, and reagents—

Our diverse collection of antibodies, assays, beads, and buffers support leading research areas.

Antibodies—

Over 10,000 conjugated antibodies are specifically developed, validated*, and manufactured for flow cytometry applications. Multiple fluorophore format options are available to help you build and expand your panels as your research demands. An essential part of evaluating any biological system is the ability to mimic or inhibit relevant pathways. This can be done with the use of Invitrogen™ eBioscience™ Functional Grade antibodies for activation, blocking, or neutralization studies, as well as recombinant proteins. These are all in appropriate preservative-free buffers with extremely low endotoxin levels to prevent any nonspecific side effects.

[thermofisher.com/flowantibodies](https://www.thermofisher.com/flowantibodies)

Assays—

We offer flow cytometry assays and reagents that span a broad range of research applications and areas, including cell health and function assays as well as RNA detection assays. [thermofisher.com/flow-assays](https://www.thermofisher.com/flow-assays)

RNA detection by flow cytometry—

The Invitrogen™ PrimeFlow™ RNA Assay reveals the dynamics of RNA and protein expression within individual cells, facilitating unprecedented analysis of their correlation as cells change over time or in response to stimuli. This novel assay employs a proprietary fluorescence *in situ* hybridization (FISH) and branched DNA (bDNA) signal amplification technique, enabling simultaneous detection of as many as four RNA transcripts in a single cell using a standard flow cytometer. [thermofisher.com/primeflow](https://www.thermofisher.com/primeflow)

Beads for instrument controls and standards—

Flow cytometers are designed to perform quantitative measurements on individual cells and other particles with high precision, speed, and accuracy, making instrument calibration a critical step in flow cytometer performance and data integrity. Experimental controls for the analysis of fluorescently labeled cells include compensation, absolute cell counts, and live vs. dead cells determination.

Sample preparation buffers and reagents—

Quality data requires quality starting material. Achieve the best possible results with highly referenced sample preparation reagents for flow cytometry, including intracellular and transcription factor staining buffer sets, cell lysis and preservation assays, blocking reagents, and magnetic cell isolation beads.



Flow cytometry instrumentation—

Instruments like the Attune NxT Flow Cytometer have helped expand the boundaries of flow cytometric analysis. The Attune NxT system was developed with the goal of removing barriers to classical flow cytometry to better enable new progress in this field. This includes increased data fidelity with faster acquisition rates and improved fluidics with acoustic focusing and resistance to clogging, thereby enlarging the scope and power of flow cytometry to areas such as cancer and synthetic biology. [thermofisher.com/attune](https://www.thermofisher.com/attune)

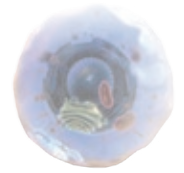


Flow cytometry education and support—

Our Thermo Scientific™ and Invitrogen™ portfolios of instruments are supported by an extensive global team of experienced scientists and engineers, who all adhere to a tenet of unrelenting reliability for service and support. We are also committed to education. Expand your understanding with our curated collection of Flow Cytometry Resource Library of technical application notes, publications, videos, webinars, and scientific posters for flow cytometry. Find out more at [thermofisher.com/flowlearning](https://www.thermofisher.com/flowlearning)

* 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.

Antibodies, assays, and reagents



Antibodies

With the addition of the eBioscience antibodies, we offer a portfolio of over 10,000 primary antibodies specifically developed, validated, and manufactured for flow cytometry applications. This catalog includes traditional and unique antibody specificities for all of your flow cytometry research needs, from the group that delivered the first commercially available antibodies for key immunology targets, including Foxp3, ROR γ t, TIGIT, CD223 (LAG3), and EOMES.

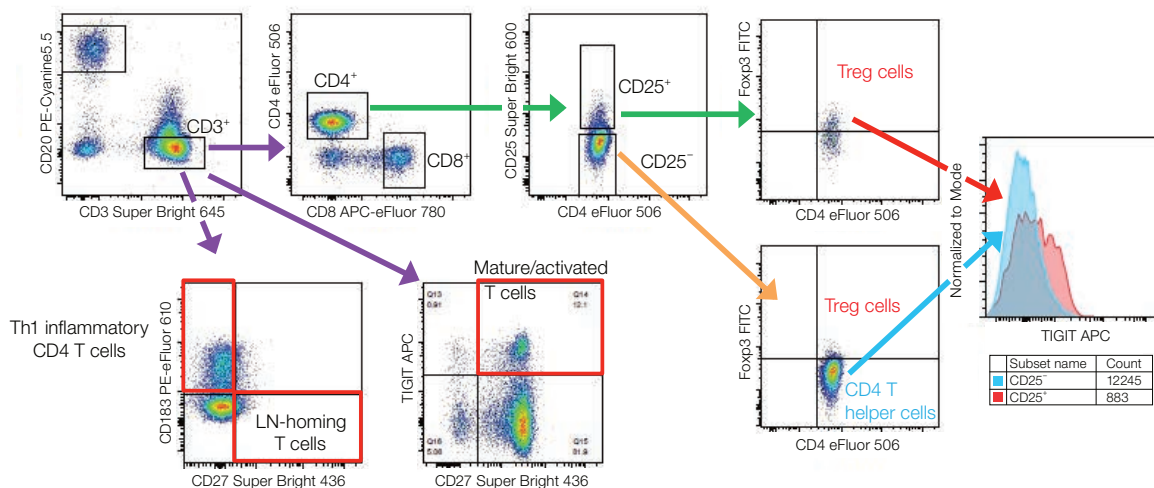
Our flow cytometry antibodies are conjugated to numerous different fluorophores to allow for flexibility, ease, and expansion of your panel needs. In addition to the traditional fluorophores such as PE, FITC, and APC, we offer three additional families of fluorophores to greater expand the depth of the flow cytometry antibody portfolio. These include Invitrogen™ Alexa Fluor™, eFluor™, and Super Bright dyes. These families of fluorophores, combined with our broad portfolio of biological content, more easily enable dye selection for optimized multicolor antibody panel design for flow cytometry.

From subset identification of heterogeneous cell populations (Figure 1) to rare-event detection, our fluorescently conjugated primary antibodies can help answer complex questions, in less time and with less sample than using other approaches. Regardless of your instrument, we have labels designed to help you get the most out of every sample and every flow cytometry run.

Search for your primary antibody conjugate at thermofisher.com/flowantibodies

Flow conjugated antibodies for:

- CD markers
- Transcription factors
- Cytokines, chemokines, and growth factors
- Phosphospecific cell signaling
- Immunology
- Immuno-oncology
- Oncology
- Apoptosis
- Cell cycle
- Stem cells



Panel:

Anti-CD3 (clone OKT3) Super Bright 645
 Anti-CD4 (clone RPA-T4) eFluor 506
 Anti-CD8a (clone RPA-T8) APC-eFluor 780
 Anti-CD20 (clone 2H7) PE-Cyanine5.5

Anti-CD25 (clone BC96) Super Bright 600
 Anti-CD27 (clone O323) Super Bright 436
 Anti-CD183 (CXCR3) (clone CEW33D) PE-eFluor 610

Anti-CD185 (CXCR5) (clone MU5UBEE) PE-Cyanine7
 Anti-Foxp3 (clone PCH101) FITC
 Anti-TIGIT (clone MBSA43) APC

Figure 1. Ten-color T cell subset panel. Human peripheral blood cells were aliquoted in the presence of Super Bright Staining Buffer, then surface-stained with the indicated reagents. Samples were then fixed and permeabilized according to the Invitrogen™ eBioscience™ Foxp3/Transcription Factor Staining Buffer Set protocol and stained with the indicated intracellular reagents. Analysis was performed to discriminate various T cell subpopulations.

Super Bright antibody conjugates

Brighter dyes for the violet laser

Featuring:

- Super Bright 436 dye
- Super Bright 600 dye
- Super Bright 645 dye
- Super Bright 702 dye
- Super Bright 780 dye
- Super Bright Staining Buffer

The Super Bright dyes are a line of bright fluorophores that are optimized for use in flow cytometry and may allow for better discrimination of dim cell populations. They allow greater utilization of the violet laser, enabling detection in the range of 436–780 nm. This provides greater signal separation, and in some cases, reduced compensation. Named according to their emission wavelengths (Figure 2), Super Bright antibody conjugates are also compatible with standard fluorophores, compensation beads, viability stains, and other flow cytometry reagents.

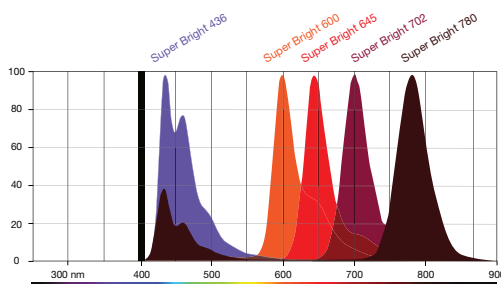


Figure 2. Emission spectra of Super Bright polymer dyes. The black bar indicates the excitation wavelength of the violet laser (405 nm).

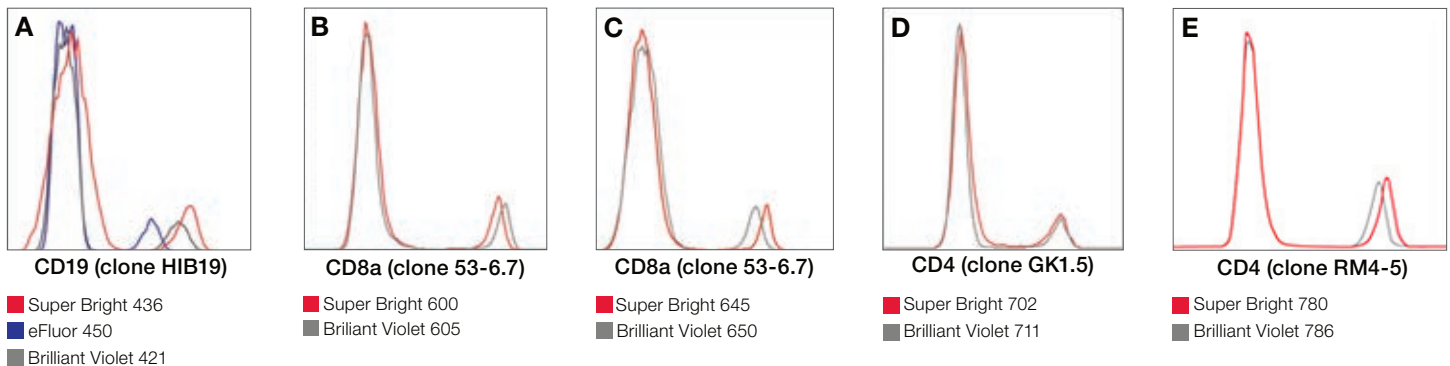


Figure 3. Fluorescence intensity comparison. (A) Human peripheral blood cells were stained with a CD19 antibody conjugated to Super Bright 436, Invitrogen™ eBioscience™ eFluor™ 450, or Brilliant Violet™ 421 dye, using the recommended volume per test. Mouse splenocytes were stained with either CD8a antibody conjugated to (B) Super Bright 600 or Brilliant Violet 605 dye or (C) Super Bright 645 or Brilliant Violet 650 dye or (D) CD4 antibody conjugated to Super Bright 702 or Brilliant Violet 711 dye or (E) Super Bright 780 or Brilliant Violet dye. For (B–D), all experiments were conducted at the same antibody concentration.

Advantages of Super Bright conjugates

- Provide improved detection of low-abundance antigens
- Allow for more data acquisition using the violet laser, freeing up other channels
- In some cases, require less compensation and have less dye–dye interaction than comparable products
- Optimal choice for multicolor flow cytometry, as they can have lower background than reagents from other suppliers
- Minimal fluorescence loss when exposed to formaldehyde fixative solution or ambient light

Stability studies

Stability studies indicate that all Super Bright conjugates exhibit minimal loss of fluorescence when cells are exposed to formaldehyde fixative for up to 3 days, or when exposed to overnight to ambient light.

Comparative data

Super Bright antibody conjugates have been tested and compared to other fluorophores for the violet laser, and in many instances, provide superior brightness (Figure 3).

Stimulation

An essential part of evaluating any biological system is the ability to mimic or inhibit relevant pathways, both at the cellular and tissue level and in whole-organism models using antibodies, proteins, or small molecules. We offer an expansive list of high-quality cell stimulation products that include Functional Grade antibodies, recombinant proteins, and ready-to-use stimulants (e.g., Invitrogen™ eBioscience™ Cell Stimulation Cocktails (PMA and Ionomycin), Con A, PHA-L) as well as Invitrogen™ Dynabeads™ magnetic beads for T cell activation and expansion (Figure 4). Functional Grade antibodies are used for activation (e.g., anti-CD3 and anti-CD28), blocking (e.g., anti-PD-1 and anti-TIGIT), or neutralization (e.g., anti-IL-2 and anti-IFN-gamma) studies. These reagents are provided in appropriate preservative-free buffers with extremely low endotoxin levels.

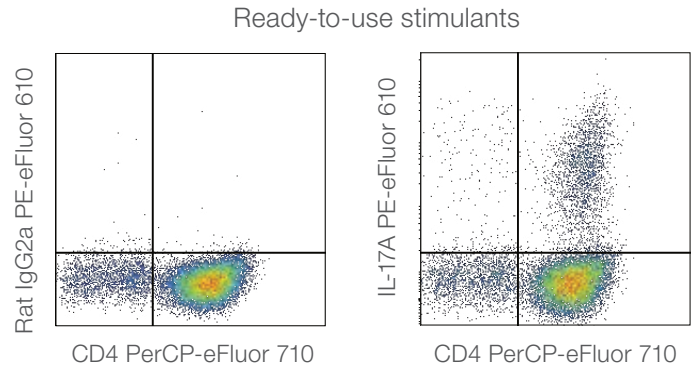
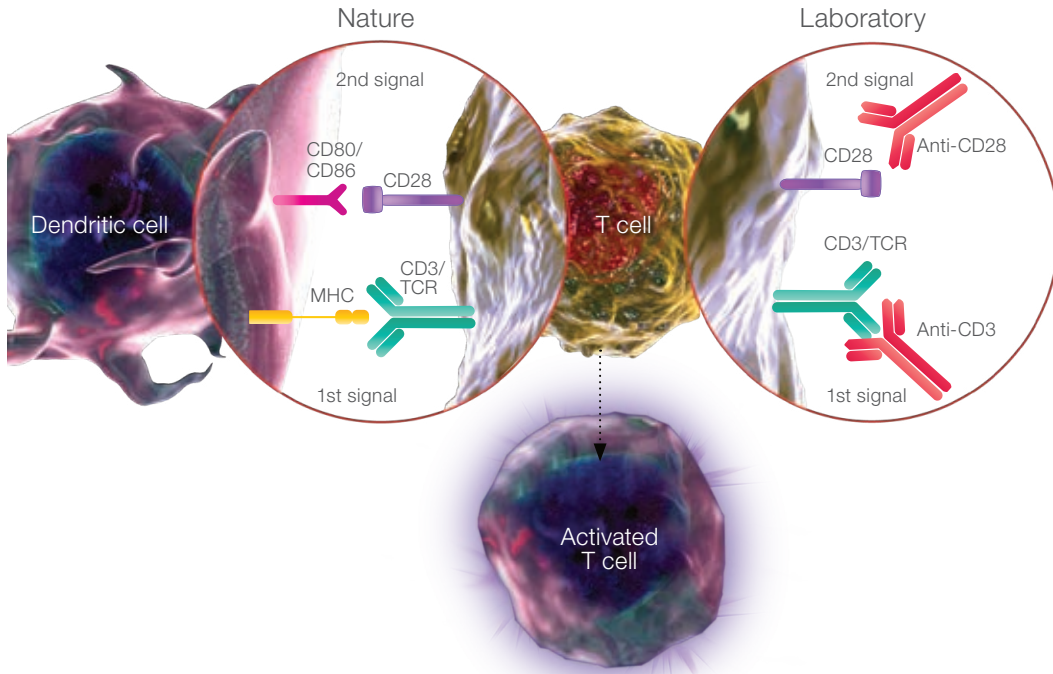


Figure 4. Splenocytes were cultured under Th17-polarizing conditions for 6 days then restimulated for 5 hours with the Cell Stimulation Cocktail (plus protein transport inhibitors). Cells were intracellularly stained with Anti-Mouse CD4 PerCP-eFluor 710 Antibody and Rat IgG2a K Isotype Control PE-eFluor 610 Antibody (left) or Anti-Mouse/Rat IL-17A PE-eFluor 610 Antibody (right) using the Intracellular Fixation & Permeabilization Buffer Set and protocol. Viable cells, as determined by an Invitrogen™ fixable viability dye in the lymphocyte gate, were used for analysis.

T cell activation



Assays



Apoptosis

Understanding the mechanisms of cell death and survival can represent a critical aspect of toxicological profiling and drug discovery.

Invitrogen™ reagents and assays are designed to effectively study changes in the plasma membrane, mitochondria, caspase activity, and DNA fragmentation and chromatin condensation as a result of apoptosis. For information about our apoptosis assays for flow cytometry, go to thermofisher.com/flow-apoptosis



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 to avoid false-positive results (Figure 5). We offer a selection of Invitrogen™ viability dyes (Table 1) and assays to choose from:

- Fixable viability dyes
- Nonfixable viability dyes
- Bacterial viability and vitality assays
- Yeast viability and vitality assays

Learn more about our viability assays at thermofisher.com/flow-cellviability



Cell cycle

Flow cytometry, in conjunction with modeling algorithms, provides a powerful tool to assess cells in the G_0/G_1 phase of the cell cycle versus

the S phase and G_2/M phase, or to analyze polyploidy. Invitrogen™ fluorescent dyes allow accurate cell cycle analysis in either live or fixed cell populations (Table 1).

For more information about our cell cycle products, go to thermofisher.com/flow-cellcycle



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 the latest technology for measuring new DNA synthesis (Invitrogen™ Click-iT™ Plus EdU labeling kits), as well as reagents for assessing cell proliferation by dye dilution (Invitrogen™ CellTrace™ cell proliferation kits, Table 1).

For more information on cell proliferation, go to thermofisher.com/flow-cellproliferation

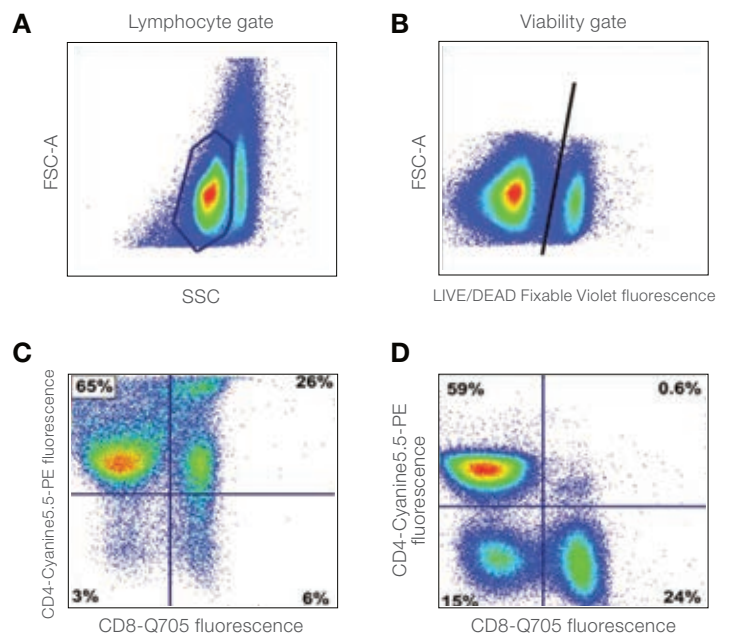


Figure 5. Exclusion of dead cells eliminates staining artifacts from analysis. After the application of a lymphocyte gate (A), live and dead cells were discriminated (B) using the LIVE/DEAD Fixable Violet Dead Cell Stain Kit. Note the significant number of dead cells despite a scatter gate. Subsequent 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.

RNA detection by flow cytometry

Expanding the capability of flow cytometry to measure RNA

Flow cytometry, with its ability to acquire and analyze millions of individual cells simultaneously, use multiplexing capabilities, and to detect both cell-surface and intracellular proteins in a straightforward workflow, has long been the standard for characterizing heterogeneous cell populations. Nevertheless, flow cytometry has been constrained by the availability and adequacy of antibodies to measure all analytes of interest. There are many areas of research such as microRNA, viral transcripts in infected cells, and antibody development in unique model organisms that have not been able to utilize the power of flow cytometry and have historically required numerous disconnected experiments by the researchers to analyze these cellular systems.

With the novel PrimeFlow RNA Assay, scientists can now reveal the dynamics of RNA and protein expression simultaneously within millions of single cells (Figure 6). This assay employs a proprietary FISH and branched DNA (bDNA) amplification (Figure 7) technique for simultaneous detection of up to four RNA transcripts labeled with 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.

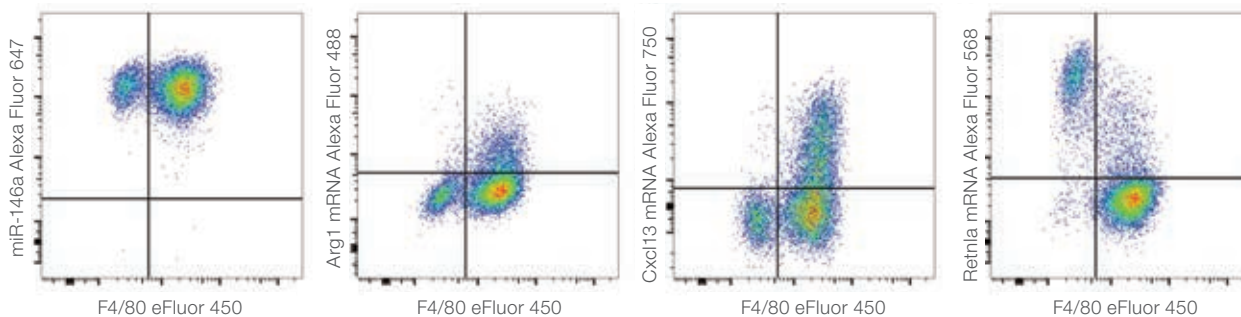


Figure 6. PrimeFlow RNA Assay detection of miR-146a, Arg1 mRNA, Cxcl13 mRNA, and Retn1a mRNA in mouse peritoneal cells.

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- α) mRNA, whereas LPM were positive for Cxcl13 mRNA and expressed low levels of Arg1 mRNA.

Novel product applications:

- Unmask gene expression heterogeneity at the single-cell level
- Correlate RNA and protein levels in the same cell
- Detect noncoding RNA, including microRNA, in cellular subsets
- Evaluate viral RNA in infected cells
- Analyze mRNA expression when antibody selection is limited

New features:

- Analyze up to four RNA transcripts simultaneously
- Detect microRNA (miRNA) by flow cytometry

Assay technology

FISH is a powerful technique that allows specific localization of RNA targets in fixed cells. The basic premise of the application relies on detecting nucleic acids through sequential hybridization of nucleic acid probes that provide gene expression information at the single-cell level. Traditional FISH techniques are generally limited by high background and low sensitivity due to nonspecific binding and inefficient signal amplification.

The PrimeFlow RNA Assay incorporates a proprietary oligonucleotide probe set design and bDNA signal amplification technology to analyze RNA transcripts by flow cytometry. bDNA technology provides a unique approach to RNA detection and signal amplification by amplifying the reporter signal rather than the target sequence (e.g., PCR) to help enable more consistent results than those obtained using PCR-based assays.

Principle of the PrimeFlow RNA Assay

Development of the PrimeFlow RNA Assay is based upon proven and well-published ViewRNA™ technology designed for microscopic analysis of RNA in cells and tissues. The assay combines paired oligonucleotide probe design with bDNA signal amplification to robustly detect up to four RNA transcripts at the single-cell level using a standard flow cytometer.

In the PrimeFlow RNA Assay, target-specific probe sets contain 20–40 oligonucleotide pairs that hybridize to the target RNA transcript. Signal amplification is achieved through specific hybridization of adjacent oligonucleotide pairs to bDNA structures, formed by pre-amplifiers, amplifiers, and fluorochrome-conjugated label probes, resulting in excellent specificity, low background, and high signal-to-noise ratios (Figure 8).

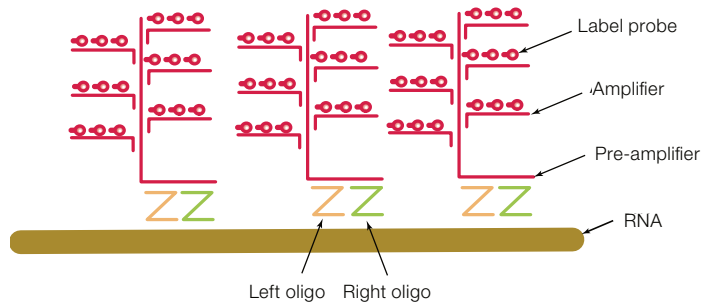


Figure 7. PrimeFlow branched DNA illustration.

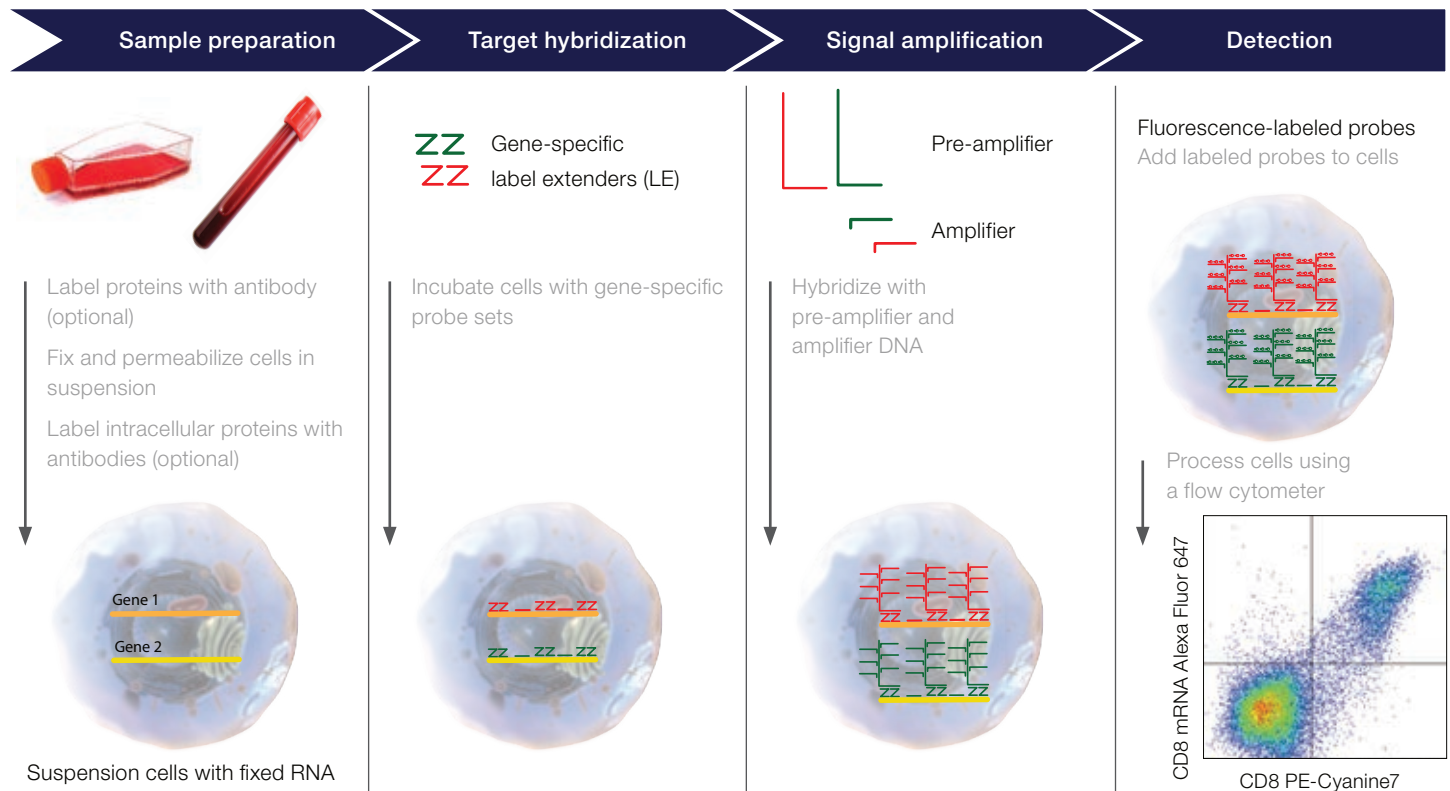


Figure 8. The PrimeFlow RNA Assay workflow. The assay workflow contains several steps: antibody staining; fixation and permeabilization, including intracellular staining, if desired; followed by target hybridization with a target-specific probe set containing 20–40 oligonucleotide pairs.

Table 1. Fluorophore and reagent selection guide for flow cytometry.

Excitation laser	Common emission filters (nm)	Attune NxT channel (nm)*	Recommended dyes	Viability dyes (compatible with fixation)	Viability dyes (unfixed cells)	DNA content/ cell cycle dyes (live cells)	DNA content/ cell cycle dyes (fixed cells)	Apoptosis dyes	Cell proliferation dyes	Reactive oxygen species (ROS) dyes	Phagocytosis dyes	Fluorescent proteins	Other dyes
UV (~350 nm)	379/28	NA											BD Horizon BUV395
	440/40 450/50	NA	Alexa Fluor 350 Alexa Fluor 405	LIVE/DEAD Fixable Blue	DAPI SYTOX Blue	Hoechst 33342 Vybrant DyeCycle Violet	FxCycle Violet (DAPI)	Annexin V, Alexa Fluor 350 Annexin V, Pacific Blue	CellTrace Blue Click-iT Plus EdU Alexa Fluor 350			ECFP	BD Horizon BUV496
	740/35	NA											BD Horizon BUV737
Violet (405 nm)	425/20 440/50 450/50 455/50 450/65 460/50	VL1 440/50 VL1(V6**) 450/40	Super Bright 436 eFluor 450 Pacific Blue Alexa Fluor 405	LIVE/DEAD Fixable Violet	DAPI SYTOX Blue	Vybrant DyeCycle Violet	FxCycle Violet	Annexin V, Pacific Blue Annexin V, eFluor 450 PO-PRO-1	CellTrace Violet Click-iT Plus EdU Pacific Blue			Azurite Cerulean TagBFP ECFP mTurquoise AmCyan	BD Horizon BV421 BD Horizon V450 VioBlue
	510/50 520/35 525/50 530/40 550/40	VL2 512/25 VL2(V6) 525/50	eFluor 506 Pacific Green	LIVE/DEAD Fixable Aqua				Violet Ratiometric Probe (F2N12S)				T-Sapphire	BD Horizon BV510 BD Horizon V500 VioGreen
	585/40 610/20	VL3 603/48 VL3(V6) 610/20	Super Bright 600 Pacific Orange Qdot 605	LIVE/DEAD Fixable Yellow				Violet Ratiometric Probe (F2N12S)					BD Horizon BV570 BD Horizon BV605 BD Horizon BV650
	660/20 660/40 710/50	VL4 710/50 VL4(V6) 660/20	Super Bright 645 Super Bright 702 Qdot 705										BD Horizon BV650 BD Horizon BV711
	710/50	VL5 710/50	Super Bright 702										BD Horizon BV711
	780/60	VL6 780/60	Super Bright 780										BD Horizon BV786
	Blue (488 nm)	525/50 525/30 525/40 530/30 530/40	BL1 530/30 BL1(G) 525/50	Alexa Fluor 488 FITC	LIVE/DEAD Fixable Green	SYTOX Green	Vybrant DyeCycle Green		Annexin V, Alexa Fluor 488 Annexin V, FITC APD-BrdU TUNEL with Alexa Fluor 488 CellEvent Caspase-3/7 Green MitoProbe DiOC ₂ (3) MitoProbe JC-1 YO-PRO-1 Iodide	CellTrace CFSE Click-iT Plus EdU Alexa Fluor 488	CellROX Green	pHrodo Green <i>E. coli</i> BioParticles Conjugate pHrodo Green <i>S. aureus</i> BioParticles Conjugate	EGFP Emerald GFP EYFP
574/26 575/30 580/23 585/40 586/15 590/40		BL2 574/26 BL2(G,Y) 590/40	PE PE-eFluor 610 PE-Alexa Fluor 610 PE-Texas Red	LIVE/DEAD Fixable Red	7-AAD Propidium Iodide (PI) SYTOX AADvanced SYTOX Orange	Vybrant DyeCycle Orange	FxCycle PI/RNase	Annexin V, PE TMRE TMRM MitoProbe JC-1			pHrodo Red <i>E. coli</i> BioParticles Conjugate pHrodo Red Phagocytosis Kit	EYFP mCitrine Venus	BD Horizon PE-CF594 PE/Dazzle 594
670/30 675/30 680/30 695/30 695/40 710/50		BL3 695/40	PerCP-eFluor 710 PE-Alexa Fluor 700 PE-Cy ⁵ .5 PerCP PerCP-Cy ⁵ .5 Qdot 705 TRI-COLOR PE-Cy ⁵		7-AAD Propidium Iodide SYTOX AADvanced			Annexin V, PerCP-eFluor 710					PerCP-Vio700
750 LP 775/50 780/40 780/60		BL4 780/60	PE-Cy ⁷ Qdot 800			Vybrant DyeCycle Ruby		Annexin V, PerCP-eFluor 710					PE-Vio770

Excitation laser	Common emission filters (nm)	Attune NxT channel (nm)*	Recommended dyes	Viability dyes (compatible with fixation)	Viability dyes (non-fixed cells)	DNA content/ cell cycle dyes (live cells)	DNA content/ cell cycle dyes (fixed cells)	Apoptosis dyes	Cell proliferation dyes	ROS detection dyes	Phagocytosis dyes	Fluorescent proteins	Other dyes
Green (632 nm)	555/20 575/24 585/16 585/42 610/20	GL1 575/36	Alexa Fluor 532 Alexa Fluor 555 Qdot 565 Qdot 605 R-phycoerythrin (R-PE, PE)		SYTOX Orange	Vybrant DyeCycle Orange		Annexin V, R-PE Annexin V, Alexa Fluor 555 MitoTracker Orange CMTMRos	CellTrace Yellow	CellROX Orange	pHrodo Red <i>E. coli</i> BioParticles Conjugate	DsRed tdTomato YFP	
	610/20 620/15 630/30	GL2 620/15	Alexa Fluor 568 Alexa Fluor 594 Qdot 605 PE-Texas Red	LIVE/DEAD Fixable Red	SYTOX AADvanced Propidium Iodide	Vybrant DyeCycle Orange	FxCycle PI/RNase	Annexin V, Alexa Fluor 568 Annexin V, Alexa Fluor 594 MitoTracker Red CMXRos	Click-IT Plus EdU Alexa Fluor 594			mCherry	PE/Dazzle 594
	695/40 710/50	GL3 695/40	Qdot 705 PE-Alexa Fluor 700 TRI-COLOR PE-Cy ⁵ 5		SYTOX AADvanced	Vybrant DyeCycle Ruby							
	780/60	GL4 780/60	Qdot 800 PE-Cy ⁷ 7										
Yellow (661 nm)	585/40 585/15 590/40	YL1 585/16	Alexa Fluor 555 PE		Propidium Iodide SYTOX Orange	Vybrant DyeCycle Orange		MitoTracker Orange CMTMRos MitoTracker Red CMXRos TMRE TMRM	CellTrace Yellow	CellROX Orange	pHrodo Red <i>E. coli</i> BioParticles Conjugate	mOrange RFP	
	610/20 613/20 615/50 620/15 620/30	YL2 620/15	PE-eFluor 610 PE-Alexa Fluor 610 PE-Texas Red	LIVE/DEAD Fixable Red	7-AAD Propidium Iodide SYTOX AADvanced SYTOX Orange	Vybrant DyeCycle Orange	FxCycle PI/RNase	Annexin V, Alexa Fluor 568 Annexin V, Alexa Fluor 594	Click-IT Plus EdU Alexa Fluor 594			DsRed mCherry mKate mStrawberry tdTomato	ECD BD Horizon PE-CF594 PE/Dazzle 594 PE-Vio 615
	670/30 675/30 680/30 695/30 695/40	YL3 695/40 YL3(B ¹ /Y/R ¹ /V6)	PE-Alexa Fluor 700 PE-Cy ⁵ 5 Qdot 705 TRI-COLOR		7-AAD Propidium Iodide SYTOX AADvanced							mPlum mRaspberry mNeptune	
	750 LP 780/40 780/60	YL4 780/60	PE-Cy ⁷ 7 Qdot 800			Vybrant DyeCycle Ruby							PE-Vio770
Red (637 nm)	660/20 661/20 665/20 670/14 670/30 675/30	RL1 670/14	Alexa Fluor 647 eFluor 660 APC Qdot 655 APC-Cy ⁵ 5	LIVE/DEAD Fixable Far Red	SYTOX Red		FxCycle Far Red	Annexin V, Alexa Fluor 647 Annexin V, Alexa Fluor 680 Annexin V, APC MitoProbe DiIC ₁ (5) TO-PRO-3	CellTrace Far Red Click-IT Plus EdU Alexa Fluor 647	CellROX Deep Red			
	720/30 730/45	RL2 720/30	Alexa Fluor 680 Alexa Fluor 700 APC-Alexa Fluor 700 Qdot 705										
	750 LP 775/50 780/40 780/60	RL3 780/60	APC-eFluor 780 APC-Alexa Fluor 750 APC-Cy ⁷ 7	LIVE/DEAD Fixable Near-IR		Vybrant DyeCycle Ruby							BD APC-H7 APC-Vio770

* Filter sets change depending on the configuration of the Attune NxT Flow Cytometer in use.

** V6 is the Attune NxT violet 6-channel configuration option.

† G refers to the green laser option.

‡ Y refers to the yellow laser option.

§ B refers to the blue laser option.

†† R refers to the red laser option.

Beads for instrument controls and standards

Compensation beads

For proper interpretation of collected data, it is important to know that the fluorescence signal being recorded for your dye of choice comes from that particular dye, and not from another dye that happens to emit some light in the same wavelength range. Capturing the correct emission data is aided by proper compensation (Figure 9). Invitrogen™ eBioscience™ UltraComp eBeads compensation beads provide options to suit your flow cytometry antibody compensation needs (Table 2 and Figure 10). The Invitrogen™ ArC Amine Reactive Compensation Bead Kit is available for compensating the LIVE/DEAD Fixable Dead Cell Stains and the Invitrogen™ GFP BrightComp eBeads Compensation Beads provide a consistent, accurate, and simple-to-use reagent for setting flow cytometry compensation when using green fluorescent protein (GFP) (Table 2).

Compensation beads are suitable for the following scenarios:

- Sample cells are in limited supply for making compensation
- Signal compensating of a positive population is needed
- Performing experiments where the panels use fluorophores whose emissions patterns overlap

Learn more at thermofisher.com/flow-compensation

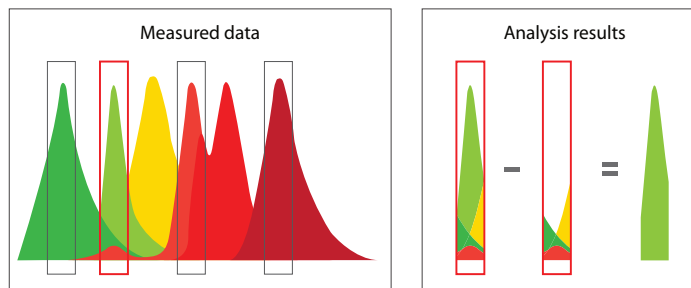


Figure 9. Spectral overlap is eliminated by compensation.

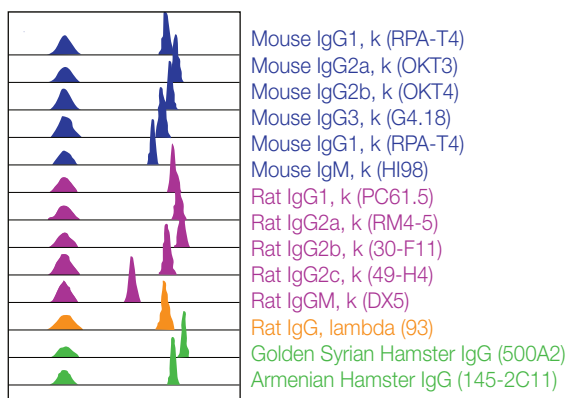


Figure 10. Staining of UltraComp eBeads with 13 different eFluor 450 dye-conjugated monoclonal antibodies including one of each subclass commonly used in flow cytometry. Beads were stained with 0.25 µg of each antibody and analyzed by flow cytometry. Each histogram represents one staining antibody (clone and isotype indicated at right).

Table 2. Invitrogen™ and eBioscience™ flow cytometry compensation bead products.

	UltraComp eBeads	GFP BrightComp eBeads™ Compensation Beads	ArC™ Amine Reactive Compensation Bead Kit
Reactivity	Hamster, mouse, and rat antibodies, and recognition of the kappa and lambda chains	Near-identical, spectral match to GFP	LIVE/DEAD™ fixable dead cell stains
Format	One vial, dispense as a single drop		1 vial positive beads 1 vial negative beads
Laser compatibility	Compatible with most standard lasers, UV to 633 nm	Designed for use with 488 nm (blue) lasers	Compatible with most standard lasers, UV to 633 nm

Calibration and size reference beads

Instrument calibration is critical to collecting and analyzing accurate experimental data. Our flow cytometer alignment and cell sorting beads are designed to help ensure your flow cytometer is performing at its peak, your experimental design is robust, and the data you collect and analyze are accurate. In addition, our size calibration and size reference kits serve as reliable size references for flow cytometer users.

- **Confidence**—ensure the reliability of optimal daily instrument performance
- **Reliability**—minimal variation for consistent data acquisition
- **Compatibility**—full range of tools for any instrument

For more information, go to [thermofisher.com/flowcalibration](https://www.thermofisher.com/flowcalibration)

Cell counting beads

Absolute cell counts have been widely used in quantifying cell concentration or absolute count of cells in a sample. We offer two cell counting products: Invitrogen™ CountBright™ Absolute Counting Beads and AccuCheck™ Counting Beads (Figure 11). They are both designed to work on any sample type, and the AccuCheck beads also provide a pipetting accuracy check.

Find out more at [thermofisher.com/flow-cell-counting-beads](https://www.thermofisher.com/flow-cell-counting-beads)

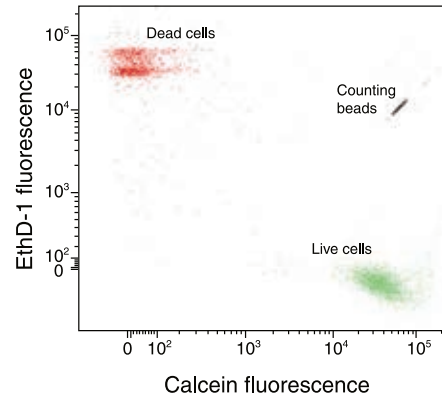


Figure 11. CountBright Absolute Counting Beads. A mixture of live and heat-killed Jurkat cells were treated with reagents in the LIVE/DEAD Viability/Cytotoxicity Kit. CountBright Absolute Counting Beads were added to the sample, which was then analyzed by flow cytometry using 488 nm excitation. Calcein 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.

Sample preparation buffers and reagents

The selection of fixation and permeabilization buffer systems has a significant impact on the quality and accuracy of the data when performing intracellular staining for flow cytometric analysis. It is important to consider the location of the target proteins within a cell to select the appropriate buffer. We provide buffer sets that are optimized for detection of nuclear proteins such as transcription factors, as well as cytoplasmic and secreted proteins. To find out more about choosing the appropriate buffer system, go to thermofisher.com/flow-sample.

Cytoplasmic staining

Cytoplasmic staining requires fixation to crosslink proteins and stabilize the cell membrane, followed by permeabilization to allow antibodies access to intracellular antigens, thereby preventing cell contents from escaping once the cell has been permeabilized. Intracellular fixation and permeabilization buffers are ideal for optimal detection of cytoplasmic proteins, including cytokines and other secreted proteins. The Invitrogen™ FIX & PERM™ Cell Permeabilization Kit is designed for use when staining proteins, such as adaptor proteins β -catenin, actin, and tubulin, in addition to receptor proteins in which the antibody recognizes a cytoplasmic version (CD152 (CTLA-4)) or cytoplasmic domain.

Secreted protein: cytokine, chemokine, and growth factors

Cytokines, chemokines, and growth factors can pose additional difficulties during evaluation by flow cytometry. These proteins go through a secretory pathway; and as such, they require protein accumulation in addition to fixation and permeabilization for optimal detection in flow cytometry analysis. This can be accomplished by blocking protein movement to different organelles and secretion through the use of transport inhibitors. This is often achieved with brefeldin A, which blocks transport at the endoplasmic reticulum; or with monensin, which blocks transport at the Golgi apparatus. Invitrogen™ eBioscience™ Cell Stimulation Cocktails contain the stimulants PMA and ionomycin, with or without the inclusion of protein transport (secretory pathway) inhibitors at a ready-to-use concentration (Figure 12).

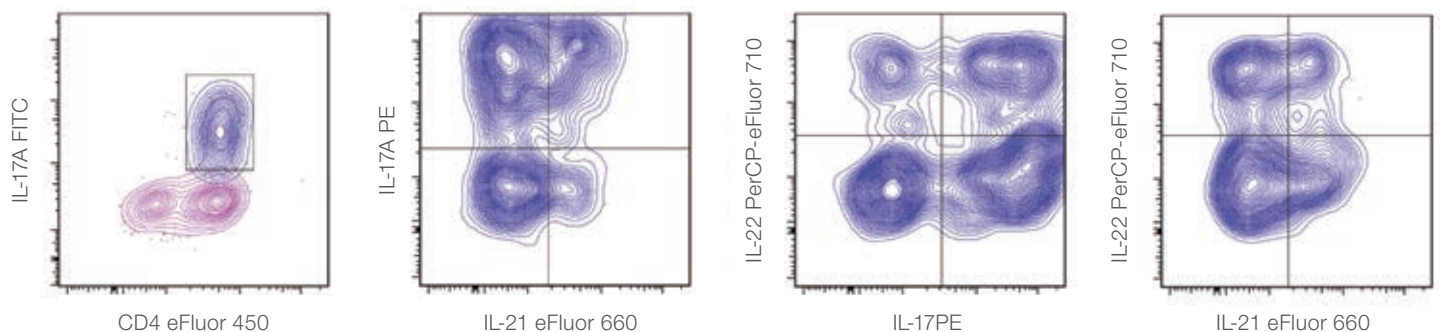


Figure 12. Human Th17 cytokine staining panel in CD4⁺ T cells. Th17-polarized CD4⁺ PBMCs were stimulated for 5 hours with Cell Stimulation Cocktail (plus protein transport inhibitors) and then intracellularly stained with the Invitrogen™ eBioscience™ Human Th17 Cytokine Staining Panel. Lymphocytes were gated for staining of CD4 eFluor 450 dye and IL-17A FITC (left plot, blue) and then analyzed for staining of IL-17F PE, IL-21 eFluor 660, and IL-22 PerCP-eFluor 710.

Nuclear staining

Transcription factors are DNA-binding proteins that regulate gene expression. Detection of transcription factors in populations at low frequencies can be challenging if the appropriate buffers are not utilized. Fortunately, the Foxp3/Transcription Factor Staining Buffer Set (although originally developed for Foxp3 staining) has been optimized for use with nuclear factors, cytosolic proteins, secreted proteins, and transcription factors, in addition to cytokines. These include Eomes, T-bet, Gata-3, IRF4, phospho-H2Ax, Ror γ (t), Egr2, Ki-67, and Sox2 (Figure 13). Additionally, this buffer has been proven to work successfully when co-staining with many secreted proteins such as IL-17A and granzyme B.

Blocking Fc-mediated nonspecific antibody binding

High background reduces data quality, making analysis difficult; but by using human, canine, or rhesus Fc receptor-blocking reagents or an anti-mouse CD16/32 antibody, optimal staining and signal-to-noise ratios can be achieved. Fc-blocking reagents are used to inhibit unwanted and nonspecific binding of antibodies to Fc receptor (Fc γ R), thereby allowing optimal staining and signal-to-noise ratios during flow cytometric analysis. To find the blocking reagent or antibody that you need, go to [thermofisher.com/flowantibodies](https://www.thermofisher.com/flowantibodies).

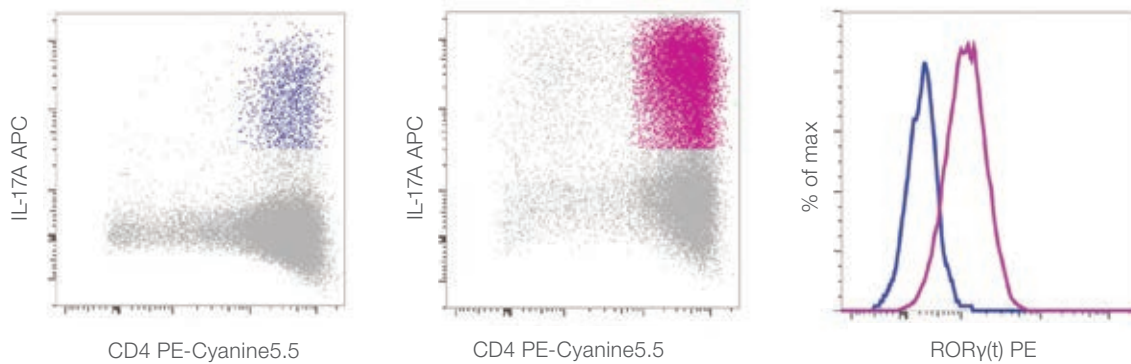


Figure 13. Identification of Th17 cells by flow cytometric detection of ROR γ (t). CD4⁺ T cells were sorted from ROR γ (t)-deficient (left plot) or wild-type (middle plot) mouse spleen and lymph nodes, cultured in Th17-polarizing conditions for 3 days, and stained with Invitrogen™ Anti-Mouse CD4 PE-Cyanine5.5, Anti-Mouse IL-17A APC, and Anti-Human/Mouse ROR γ (t) PE. The histogram shows staining of ROR γ (t) in CD4⁺/IL-17A⁺-gated events from ROR γ (t)-deficient mice (blue line) and wild-type mice (pink line) (right plot). Cells in the lymphocyte gate were used for analysis. Data provided courtesy of Dr. Littman, New York University.

Instrumentation

Attune NxT Flow Cytometer and Autosampler

Speed isn't just about going fast; it's about enabling discovery

Run samples faster and achieve greater resolution—with little fear of sample loss due to clogging. The Attune NxT Flow Cytometer with Autosampler combines precision with performance in a benchtop flow cytometer that is configurable with up to four lasers and 16 parameters of detection (Table 3). Do more. Find more. Explore more.

- **Transform your research**—get a superior level of data fidelity at speeds 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
- **Enable new applications and sample types**—high sample processing rates make it possible to evaluate difficult sample types (such as tumors) that you may not have been able to investigate before
- **Expand your capabilities**—system modularity with up to four lasers and 16 detection channels allows you to upgrade as your research needs expanding
- **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
- **Run reliably for hours at a time**—The Attune NxT instrument is available with robotic automation.
- **Microplate taxiing**—automated with extended unmanned runtime settings, scalable configuration flexibility, and maximized capacity for processing staggering quantities of data

Learn more at thermofisher.com/attune



“We have yet to clog the machine with our debris-rich primary tumor samples. Of course, the acoustic technology greatly facilitates the identification of small populations, like cancer stem cells, increasing our capacity to detect and quantify these rare events with high efficiency and reliability.”

– Bruno Sainz Jr, PhD, Autonoma University of Madrid, School of Medicine

“I knew I had learned a lot during 25 years of experience doing research with flow cytometry. Now I am surprised to see how much I can learn doing research with the Attune NxT (instrument), and how this new technology can be very helpful to make visible the invisible.”

– Dr. Jordi Petriz, PI at Josep Carreras Leukaemia Research Institute, Barcelona, Spain; executive board member at the European Society for Clinical Cell Analysis (ESCCA)

“We looked at several metrics and compared the Attune NxT Autosampler to other 96-well plate readers. The Autosampler proved to have very good stability and very low carryover. We were most impressed by the way that the autosampler took advantage of the Attune NxT Flow Cytometer’s fluidics and high-volume throughput. Without compromising stability or precision, the Autosampler was able to run plates much faster than any other plate reader.”

– E. M. Myer, University of Pittsburgh Cancer Institute

Table 3. Laser configurations and antibody options for the Attune NxT Flow Cytometer.

Excitation laser	Channel	Antibody labels	Blue	Blue/violet	Blue/violet 6	Blue/green	Blue/red	Blue/yellow	Blue/red/violet	Blue/red/violet 6	Blue/violet/yellow	Blue/violet/green	Blue/red/green	Blue/red/yellow	Blue/red/yellow/violet	Blue/red/yellow/violet 6	Blue/red/violet/green		
Violet (405 nm)	VL1 (440/50) (450/40)*	Super Bright 436																	
		Alexa Fluor 405																	
		eFluor 450		•	•*					•	•*	•	•			•	•*	•	
		Pacific Blue																	
	VL2 (512/25) (525/50)*	eFluor 506		•	•*					•	•*	•	•			•	•*	•	
		Pacific Green																	
	VL3 (603/48) (610/20)*	Super Bright 600			•	•*				•	•*	•	•			•	•*	•	
Pacific Orange																			
VL4 (710/50) (660/20)*	Super Bright 645			•	•*				•	•*	•	•			•	•*	•		
VL5 (710/50)	Super Bright 702				•					•						•			
VL6 (780/60)	Super Bright 780				•					•						•			
Blue (488 nm)	BL1 (530/30) (525/50)*	FITC																	
		Alexa Fluor 488	•	•	•	•*	•	•	•	•	•*	•*			•	•	•	•*	
	BL2 (590/40) (574/26)*	PE																	
		PE-Alexa Fluor 610	•*	•*	•*			•*		•*	•*	•	•		•*	•	(695/40)	•	
		PE-Texas Red																	
	BL3 (695/40)	PE-Alexa Fluor 700																	
		PE-Cy5																	
PE-Cy5.5		•	•	•	•	•	•	•	•	•	•	•		•	•		•		
PerCP-Cy5.5																			
BL4 (780/60)	PE-Alexa Fluor 700	•	•				•		•					•					
	PE-Cy7																		
Green (532 nm)	GL1 (575/36)	Alexa Fluor 532																	
		Alexa Fluor 555					•						•	•				•	
		PE																	
	GL2 (620/15)	PE-eFluor 568																	
Alexa Fluor 594						•							•	•				•	
GL3 (695/40)	PE-Alexa Fluor 700													•	•			•	
	PE-Cy5.5																		
GL4 (780/60)	PE-Cy7					•							•	•				•	
Yellow (561 nm)	YL1 (585/16)	PE													•	•	•	•	
	YL2 (620/15)	PE-eFluor 610																	
		Alexa Fluor 568																	•
	YL3 (695/40)	PE-Texas Red																	•
PE-Alexa Fluor 700																		•	
PE-Cy5																		•	
YL4 (780/60)	PE-Cy5.5																	•	
	PE-Cy7																	•	
Red (637 nm)	RL1 (670/14)	APC																	
		eFluor 660																	
		Alexa Fluor 647						•		•	•				•	•	•	•	
	RL2 (720/30)	Alexa Fluor 680																	
		Alexa Fluor 700						•		•	•				•	•	•	•	
	RL3 (780/60)	APC-Alexa Fluor 700																	
Alexa Fluor 750																			
APC-Alexa Fluor 750							•		•	•				•	•	•	•		
APC-eFluor 780																			
	APC-Cy7																		

* Denotes Attune NxT Flow Cytometer Violet 6–channel option

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Resources

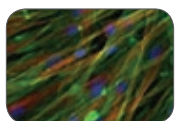
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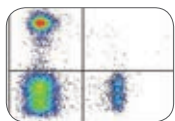
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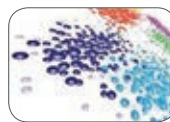


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