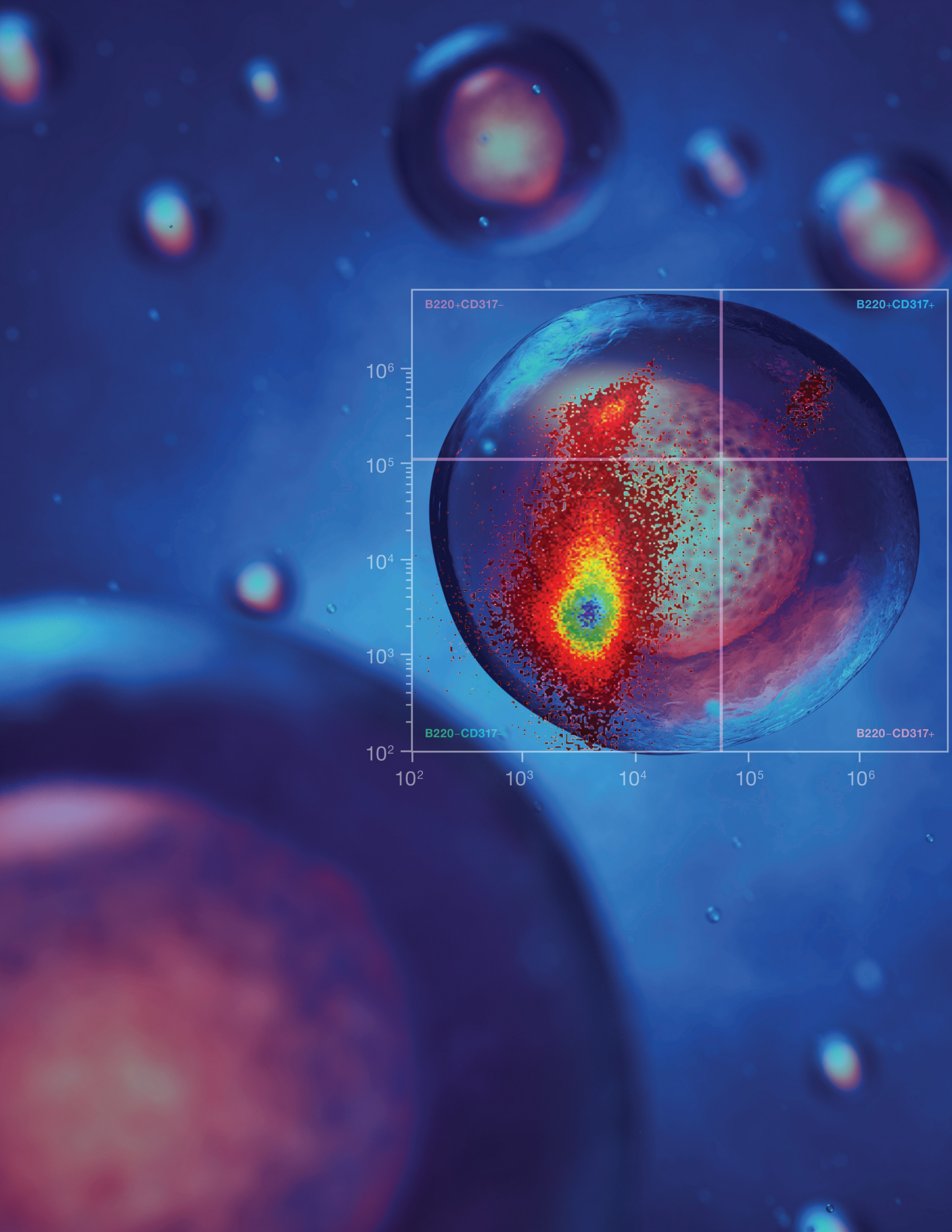
invitrogen

Empowering technologies for immuno-oncology research

Stimulation antibody | Conjugated antibodies | Cell health | RNA flow cytometry | Flow cytometry instrument

Accelerating discovery with simplified workflows for flow cytometry, biomarker profiling, and cell imaging

ThermoFisher SCIENTIFIC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Contents

Deep dive into immuno-oncology

Recent breakthroughs in immunotherapy research bring about a different treatment approach for cancer. Discoveries to create inhibitors targeting cell checkpoint proteins and methods for gene modification lead to advancement of therapeutic knowledge [1-3]. Each of these innovations and findings are important as immunotherapy aims for long-lasting tumor regression.

The field of immuno-oncology is now diving deeper and pushing the boundaries of innovation. Further research is underway to target and uncover more proteins in cell pathways. Data sets are being thoroughly analyzed to create cancer biomarker panels. Adoptive T cell therapy is expanding with the identification of better cell surface antigens for specific targeting. The ultimate goal is to harness the full capabilities of the immune system to help combat cancer.

Thermo Fisher Scientific offers many research platforms and products to help better understand the interplay between the immune system and cancer. Expand the experimental capabilities with our instruments and reagents to accelerate the development of cancer immunotherapies of tomorrow.

Tumor microenvironment

Microenvironments of solid tumors are composed of cancer cells, stromal cells, and immune cells [4]. Cells interact through direct cell–cell contact or through indirect signals such as ligands or growth factors. Each of these cells plays a role to influence each other and execute processes such as cancer progression, tumor invasion, and immune evasion [5].

Immune cells found in the microenvironment of tumors form a complex network (Table 1) [6-12]. Each cell type has a specialized function in the pursuit to recognize and eliminate tumor cells or foreign bodies (Figure 1). Recognition between normal, and pathogen or abnormal cells is regulated by a balance between co-stimulatory and inhibitory signals. Some of these signals are secreted directly by the tumor cells to provide host immunity and promote resistance to therapeutic intervention.

Table 1. Immune cell types. Effector cells directly eliminate foreign pathogens and cancer cells. Non-effector cells indirectly influence cancer cell death through modulating the cytotoxic effector T cell response.

Effector cells	Non-effector cells
Natural killer (NK) cells provide the innate immune system's rapid immunity against metastasis. NK cells' activating and inhibitory receptors scan for foreign or altered self–protein expression patterns.	Antigen presenting cells (APCs), such as dendritic cells, present antigens for T cells by displaying a major histocompatibility complex (MHC) molecule on the cell surface.
Cytotoxic T cells are effector cells for adaptive immunity. Pathogens or cells presenting non–self antigens are killed through cytotoxic-mediated apoptosis.	Regulatory T (Treg) cells inhibit the immune process through modulating T cell responses.
Memory T cells are part of the adaptive immune response, generating immunity after the pathogen response or vaccination. Memory T cells deliver long-term immunity by circulating through the body and rapidly proliferating upon detecting the antigen.	Tumor-associated macrophages (TAMs) inhibit activated immune effector cells such as T cells, B cells, and NK cells. These macrophages express inhibitory factors such as programmed cell death protein-1 (PD-1) and cytotoxic T lymphocyte–associated antigen-4 (CTLA-4).

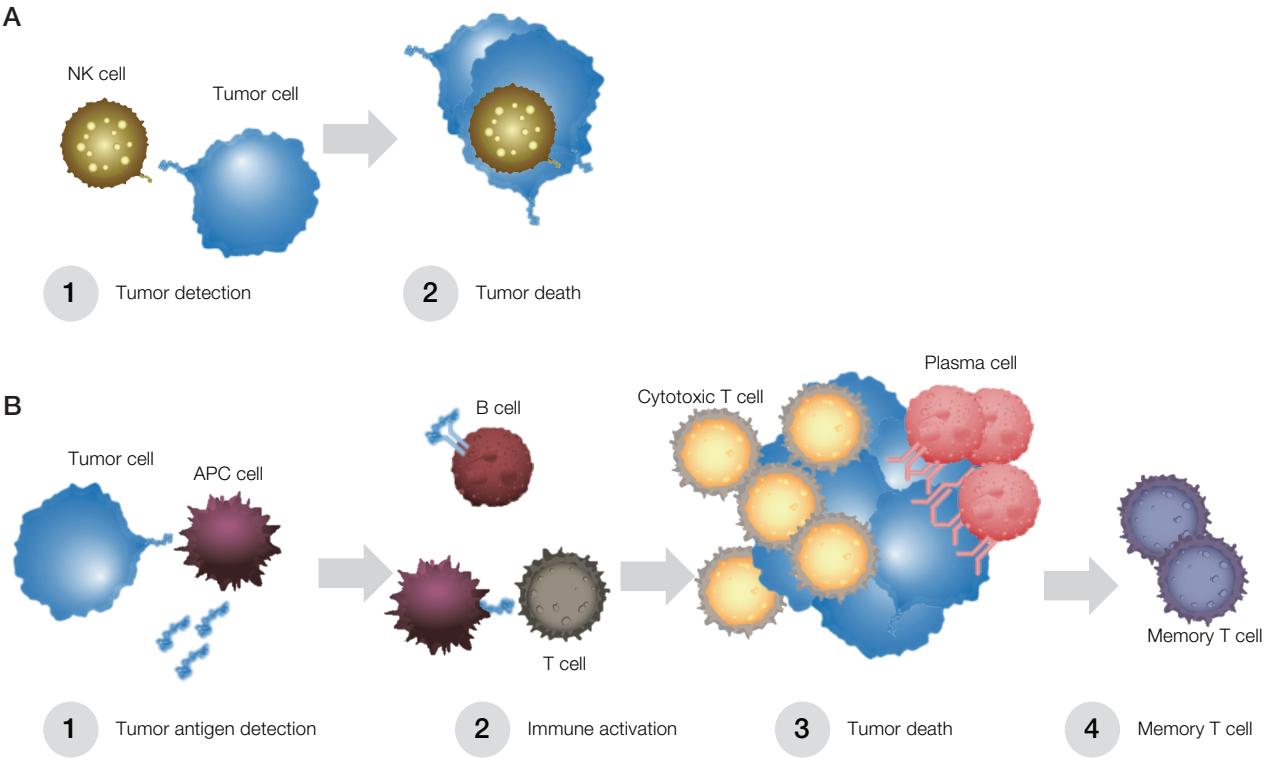


Figure 1. Interplay between tumor cells and cells of the immune system. (A) Innate immunity uses immune cells such as NK cells to directly target and destroy tumor cells. **(B)** Adaptive immunity requires APC cells to present antigens to inactive T cells and activate the immune system. This provides long-term immunity. Inhibitory cells such as TAMs can negatively influence immune activation in the tumor microenvironment.

Cell checkpoints

Immune checkpoints are cell pathways crucial in maintaining a normal immune response and protecting tissues from damage when the immune system is activated [4,5]. Tumor cells dysregulate immune checkpoints and use them as a mechanism of immune resistance. Understanding immune checkpoints in NK cells and T cells is the main focus in immuno-oncology research as these cells regulate both the adaptive and innate immunity surrounding the tumor (Table 2) [4,5,7,8,11].

Table 2. Druggable targets in immune checkpoint pathways.

Immune cell	Effector cell	Function
Cytotoxic T lymphocyte–associated antigen-4 (CTLA-4)	T cells	Tumor cells use the CTLA-4 pathway to decrease T cell activation and ability to proliferate into memory T cells
Programed cell death protein (PD-1)	T cells	PD-1 expression on T cell indicates exhaustion and inability to perform immune responses
OX40	T cells	Activating OX40 stimulates T cell differentiation and cytolytic function leading to enhanced anti-tumor immunity
Glucocorticoid-induced TNFR-related (GITR)	T cells	GITR activation enhances cell reproduction and generates antitumor activity
Indoleamine-2,3 dioxygenase (IDO)	Tregs	Tumor cells can upregulate IDO activity through the breakdown of tryptophan in order to suppress T cell function
CD73	Tregs	Tumor cells use CD73 to suppress T cell activity with the production of adenosine
Lymphocyte-activation gene (LAG)	T cells and Tregs	LAG expression leads to T cell exhaustion and inhibits long-term immune response development
CD137	T cells and NK cells	CD137 stimulates NK cells and T cells for anti-tumor response along with immune memory
SLAM family member 7 (SLAMF7)	NK cells	SLAM7 activation stimulates NK cells and other immune cells in the development of long-term immunity
Killer-cell immunoglobulin-like receptors (KIR)	NK cells	Tumor cells evade NK cells with KIR expression
Programed cell death protein ligand (PD-1)	Tumor cells	PDL-1

One of the important research goals in immuno-oncology is to achieve activation of the anti-tumor immune response through the blockade of negative regulators of the immune system. Many of these immune checkpoints are initiated by interactions between membrane-bound ligands and receptors, and can be readily blocked by antibodies targeting these ligands or receptors. Key checkpoint regulators including CTLA-4, PD-1, and PD-L1 are some of the most widely studied ones that are being developed as pharmaceutical agents. These checkpoint proteins negatively regulate the immune system in a number of cancers, including metastatic melanoma, non-small cell lung cancer (NSCLC), kidney cancer, and lymphomas. Checkpoint inhibition creates long-term durable responses in a subset of patients.

Genetically modified T cells for cancer therapy

Adoptive cell therapy (ACT) is a personalized treatment approach [13,14]. ACT is generated by genetically modifying a patient's own T cells to target antigens selectively expressed on cancer cells. Successful applications of ACT include T cells expressing receptors or chimeric antigen receptors (CAR) for cancer treatment (Figure 2).

The T cell receptor (TCR) participates in the activation of T cells [15]. Its stimulation is triggered in response to cells expressing MHC molecules with the antigen.

Tumor-specific TCRs can be genetically engineered to recognize specific cancer cell populations. TCR technology is unique as it recognizes both intracellular and cell surface proteins, conferring a broad array of antigen targets. Limitations include patient-specific human leukocyte antigen (HLA) restrictions and the lack of unique tumor-specific antigens.

CARs are fusion proteins combining intracellular T cell components and extracellular antigen-recognition domains from a monoclonal antibody [13-15]. They can be constructed by linking the variable regions of the heavy and light chains of the antibody to intracellular signaling chains (such as CD3-zeta, CD28, 41BB) with other signaling factors. T cells that are engineered to express CARs are not limited by HLAs, since a CAR molecule recognizes an intact-cell antigen on the surface of a cancer cell. However, they are limited by their inability to recognize mutated intracellular proteins.

Cytokine release syndrome (CRS)

Foreign or manipulated cells can sometimes induce a strong response from the immune system. CRS is a storm of cytokines released in a patient, creating a cascade of systemic inflammatory responses. Active research is being conducted to examine the source of the cytokines—from leukemia cells to macrophages [13].

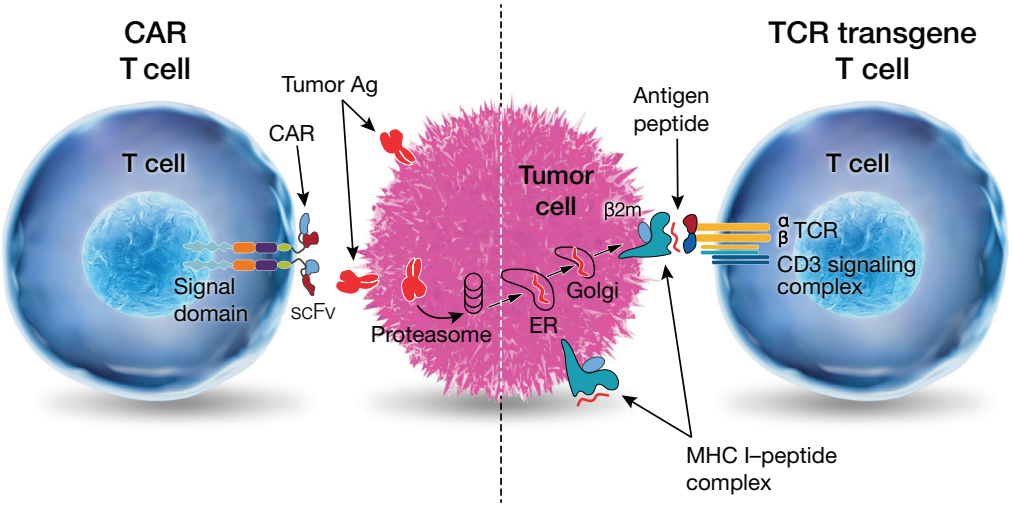


Figure 2. TCRs and CARs designed to specifically recognize and target tumor cells. Differences are found in the mechanism for antigen recognition. TCRs are composed of one α chain and one β chain, and recognize antigens that have been processed and presented by MHC molecules. CARs are constructed with a membrane-distal single-chain variable region (scFv) made of the variable heavy and light chains joined by a linker molecule.

Attune NxT Flow Cytometer

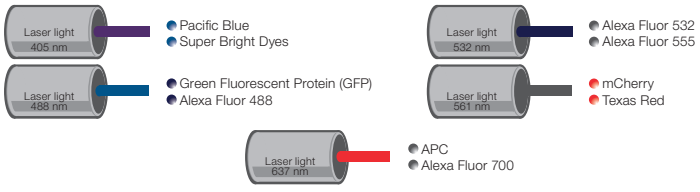
Designed for clog resistance and speed

Performance advantages of the Attune NxT Flow Cytometer

The Invitrogen™ Attune™ NxT Flow Cytometer (Figure 3) is a high-performance instrument with features, capabilities, and specifications that support the demands of researchers at the frontier of immuno-oncology for the fight against cancer. Important advantages include:

- **Highest level of data fidelity**—find the needle in the haystack up to 10x faster than traditional cytometers at a true 35,000 events/second acquisition and 1 mL/min rate with acoustic-assisted hydrodynamic focusing
- **New applications to explore difficult samples**—investigate complex samples, including digested tumor samples, without worrying about losing your precious samples, with clog-resistant engineering at ultralow coincidence and abort rates
- **Precision volumetric cell counting in a known volume**—beadless counting using volumetric sheath delivery that stabilizes laser delay, reduces temperature-based velocity variances, and enables consistent and accurate data across the full operating range
- **Extensive tool kit for compensation**—full matrix with automated and manual modes; on-plot compensation tools for fine adjustment and managing compensation of popular dye combinations
- **Simplified sample prep for optimized workflow**—able to perform immunophenotyping analysis on minimally processed samples, reducing the time-intensive, traditional 10-step sample prep protocol to 3 straightforward steps
- **Ease and flexibility**—convert between tubes and plates in seconds with the click of a mouse

Learn more at **thermofisher.com/attune**



Detect full range of fluorescence—top-of-the-line fiber-optics, flat-top lasers, up to 4 colors, and laser simmer to detect dim-to-bright signals

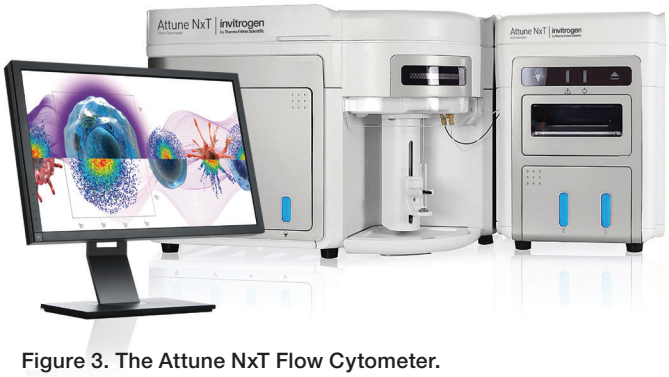
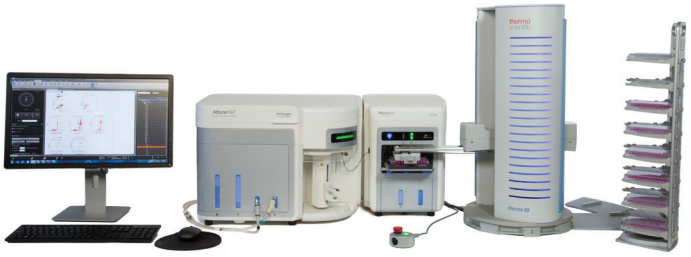
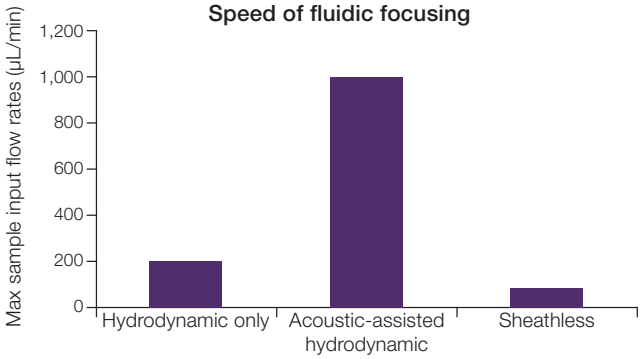


Figure 3. The Attune NxT Flow Cytometer.



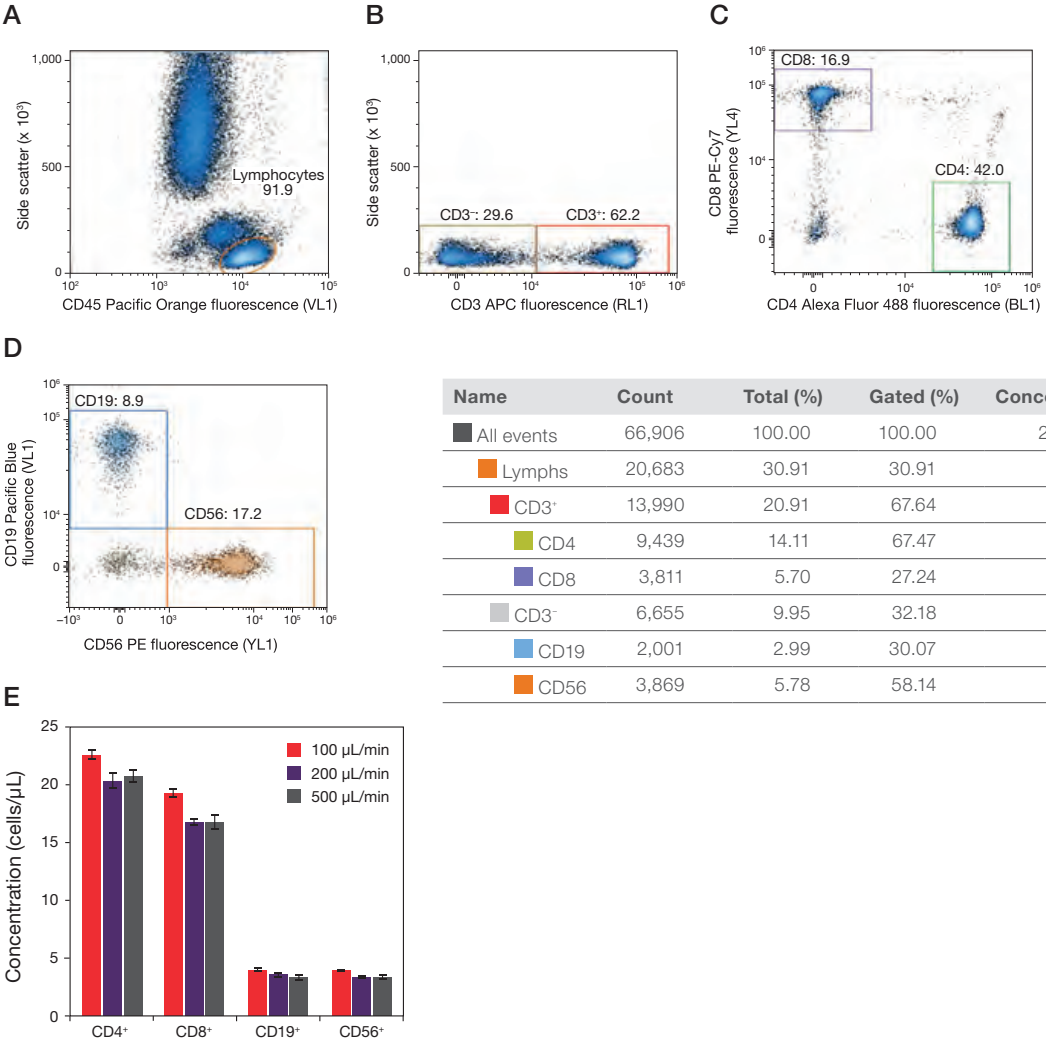
Optimize automation with robotics—compatible with the Thermo Scientific™ Orbitor™ RS Microplate Mover and temperature-stable SmartStor™ Unit for streamlined efficiency.



Name	Count	Total (%)	Gated (%)	Concentration
All events	66,906	100.00	100.00	297.4
Lymphs	20,683	30.91	30.91	91.9
CD3+	13,990	20.91	67.64	62.2
CD4	9,439	14.11	67.47	42.0
CD8	3,811	5.70	27.24	16.9
CD3-	6,655	9.95	32.18	29.6
CD19	2,001	2.99	30.07	8.9
CD56	3,869	5.78	58.14	17.2

Accurate cell counting—volumetric fluidics provides absolute cell counting without using counting beads

Quickly produce data without losing accuracy. Here is an example of examining cells from tumor and blood samples with the Attune NxT Flow Cytometer (Figure 4). The Attune NxT Flow Cytometer's volumetric fluidics and the acoustic-assisted hydrodynamic focusing system produces highly accurate cell concentration data with an increased sampling rate.



Name	Count	Total (%)	Gated (%)	Concentration
All events	66,906	100.00	100.00	297.4
Lymphs	20,683	30.91	30.91	91.9
CD3⁺	13,990	20.91	67.64	62.2
CD4	9,439	14.11	67.47	42.0
CD8	3,811	5.70	27.24	16.9
CD3⁻	6,655	9.95	32.18	29.6
CD19	2,001	2.99	30.07	8.9
CD56	3,869	5.78	58.14	17.2

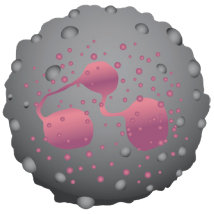
Figure 4. Lymphocyte subset analysis. A 100 µL aliquot of normal human whole blood was labeled with fluorophore-conjugated antibodies against CD surface markers, followed by red blood cell lysis using 2 mL of Invitrogen™ High-Yield Lyse Fixative-Free Lysing Solution (Cat. No. HYL250), resulting in a 1:21 dilution of the blood. **(A)** Lymphocytes are identified on a density plot of CD45 vs. side scatter with an oval gate around the lymphocyte ($CD45^+$) population. **(B)** Cells in the lymphocyte gate are displayed on a density plot of CD3 vs. side scatter. Rectangle gates surround the $CD3^+$ T cell, and $CD3^-$ B and natural killer (NK) cell populations. **(C)** Cells in the $CD3^+$ gate are then displayed on a density plot of CD4 vs. CD8 to quantify $CD4^+$ helper T cells ($CD4^+$, $CD3^+$, and $CD45^+$) and $CD8^+$ cytotoxic T cells ($CD4^+$, $CD3^+$, and $CD45^+$). **(D)** $CD3^-$ cells are displayed on a density plot of CD56 vs. CD19 to distinguish $CD56^+$ NK cells from $CD19^+$ B cells. The statistics table shows the gating and measured concentrations (cells per µL). **(E)** Replicate samples collected at three flow rates on the Attune NxT Flow Cytometer. Cell concentrations were measured using three different flow rates: 100, 200, and 500 µL/min. The Attune NxT Flow Cytometer provides similar concentration measurements for each lymphocyte subpopulation, regardless of the flow rate. Each bar represents the mean cells/µL ±standard deviation of three samples run at each indicated flow rate for each population.

Flow cytometry antibodies and reagents for studying cancer

Application spotlight

Build an optimized multiparametric panel

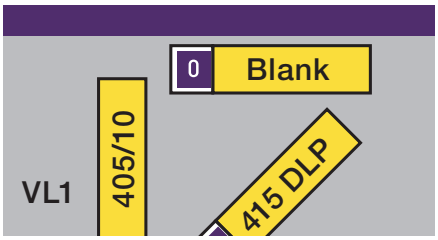
1



Marker categories:
- Lineage markers
- Checkpoint inhibitors
- Cancer stem cells

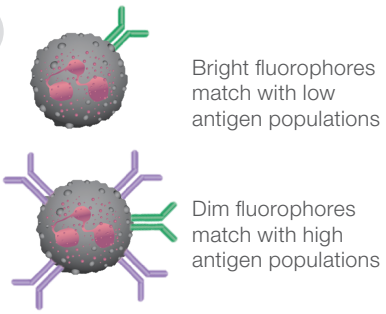
List markers to identify target cell

2



Number of fluorophores in a panel is dependent on the number of lasers and filters

3

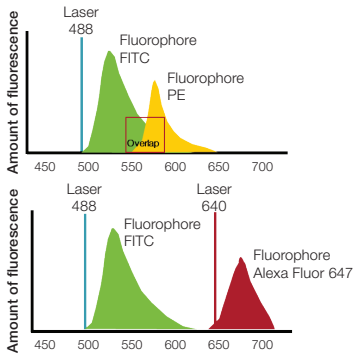


Bright fluorophores match with low antigen populations

Dim fluorophores match with high antigen populations

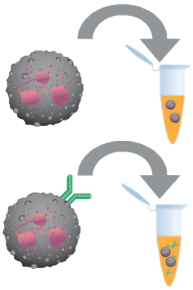
Understand the expressed antigens

4



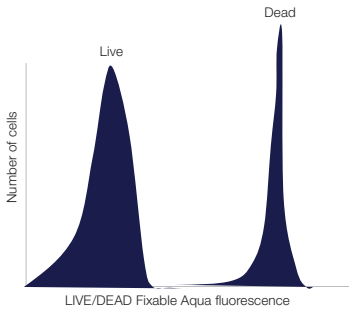
Minimize overlap of emitting fluorescence by picking separate emission spectrums

5



Include unstained control cells, single-color stain controls

6



Add live/dead dyes to only study healthy cells

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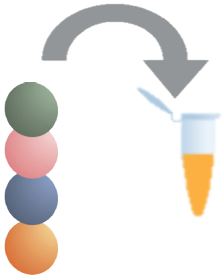
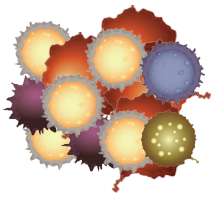


Use fluorescence compensation beads to help gate cells when there is not enough sample or poorly expressed antigens

8

Test and run your fully formed panel on the Attune NxT Flow Cytometer

Considerations in building a multiparametric fluorescence panel for flow cytometry experiments.



Work with tough samples—high-quality fluidics and a larger flow cell help prevent the loss of precious samples from clogging

Reliable service and support—full service and support for application, operation, and repair, plus complete training

Flow cytometry antibodies and functional reagents

Multiplex assays to understand more about a single cell

Study the heterogeneous cell population found in a tumor with our broad collection of reagents. From antibodies to optimized assays, the comprehensive Invitrogen™ portfolio of conjugated flow cytometry antibodies and reagents is designed to offer convenience and flexibility for research applications. It is now easier than ever to simultaneously multiplex and assay for protein levels, gene expression, and cell functions. Advantages to using reagents singly or multiplexing include:

- **Spend less time developing assays**—an extensive catalog of validated antibodies to fit into any multicolor panel or experiment
- **Generate more complex information**—reagents and antibodies can be combined to measure different parameters in the same sample
- **Expand the depth of knowledge**—expansive list of cell function assays, including cell cycle, proliferation, viability, and apoptosis

Reagent resources

	Reagent information	Highlights include:
 Cell isolation	Prepare single-cell suspensions from tumor, blood, and cultured samples. Eliminate tedious isolation and expansion steps by using innovative and high-quality reagents. To learn more, go to **thermofisher.com/cellisolation**	• State-of-the-art Invitrogen™ Dynabeads™ magnetic beads activation and expansion technology mimics *in vivo* T cell activation • Fixation kits are compatible with analysis of most cellular antigens • Proven protocols to prepare cells from both culture and *in vivo* samples
 Cell viability	Dead cells can give false-positive results as they nonspecifically bind to many reagents. Removing dead cells is a critical step for obtaining accurate flow cytometry results. Invitrogen™ cell viability reagents minimize the number of steps to measure the percentage of live and dead cells. Choose from a selection of viability dyes at **thermofisher.com/cellviability**	• A wide range of dyes spanning the spectrum and used in many fluorescence channels to fit into multicolor panels • Ability to test viability of bacteria and yeast • Fixable and non-fixable options
 Antibody	With the combined Invitrogen™ eBiosciences™ portfolio, we bring over 10,000 primary conjugated antibodies for flow cytometry use (Figure 5). The wide range of fluorochrome-conjugated antibodies identifies human, mouse, rat, or non-human primate cell antigens, and can be multiplexed with other Invitrogen™ reagents and assays. To learn more, go to **thermofisher.com/flowantibodies**	• Specifically developed, validated, and manufactured for flow cytometry applications • Large number of antibodies conjugated to popular dyes, including FITC, APC, Invitrogen™ Alexa Fluor™ dyes, eBiosciences™ eFluor™ dyes, and the recently developed family of Invitrogen™ Super Bright polymer dyes • Technical support and online tools to help build multicolor panels

Reagent resources (cont.)

	Reagent information	Highlights include:
 Cell proliferation	Proliferation measurements are typically made based on DNA synthesis or on cellular metabolism parameters. Assays can report cell health, genotoxicity, and inhibition of tumor cell growth during drug development. Invitrogen™ CellTrace™ assays (Figure 6) and Invitrogen™ Click-iT™ EdU assays (Figure 7) provide sensitive reagents to measure proliferation in many cell types. To learn more, go to **thermofisher.com/flow-cellproliferation**	• Quantitation of newly synthesized DNA • Detection without denaturation of DNA • Compatibility with sensitive R-PE tandems and fluorescent proteins • Fast detection—in as little as 60 minutes • An alternative to the cumbersome BrdU assay
 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, the mitochondria, caspase activity, and DNA fragmentation and chromatin condensation as a result of apoptosis. To learn more, go to **thermofisher.com/flow-apoptosis**	• Many assays to detect different targets found in early to late events • Range of fluorescent options to be excited from multiple laser excitation sources • Assays are compatible and used in multiplexing experiments
 RNA	Invitrogen™ PrimeFlow™ RNA assays are designed for RNA and protein expression analysis of individual cells. This novel assay employs a proprietary fluorescent *in situ* hybridization (FISH) and branched DNA (bDNA) signal amplification technique that enables the detection of up to four RNA transcripts in a single cell, and can be combined with standard flow cytometry antibody staining using standard flow cytometry. To learn more, go to **thermofisher.com/primeflow**	• Unprecedented analysis of their correlation as cells change over time or in response to stimuli • High-throughput ready with optimized multiwell kits and probes for many RNA targets • Assay for any protein when no antibody is available
 Instruments	When using two or more fluorochromes in a single experiment, some portion of the emission spectra of a given fluorochrome may fall within the detector of another fluorochrome. Simplify compensation with guided software and products to help set up proper controls.	• The Attune NxT Flow Cytometer has two different software guides to help set up for negative and single-control compensation • To calculate how much compensation is needed, single-color control samples must be run with each experiment. Beads, such as Invitrogen™ UltraComp eBeads™ or OneComp eBeads™, AbC™ Total Antibody Compensation Beads, or cells stained with the antibodies used in the experiment, can be used as single-color controls
 Panel builder	Creating assays for multiple antigens and cell functions does not need to be difficult. Multiple tools and reagents are available to help simplify design needs. To learn more, go to **thermofisher.com/readyflow**	• Field and technical support specialists to help guide through panel building and finding reagents • No pipette and titrate assays to measure cell functions such as cell viability or apoptosis • Online tools to pick fluorophores and find antibodies

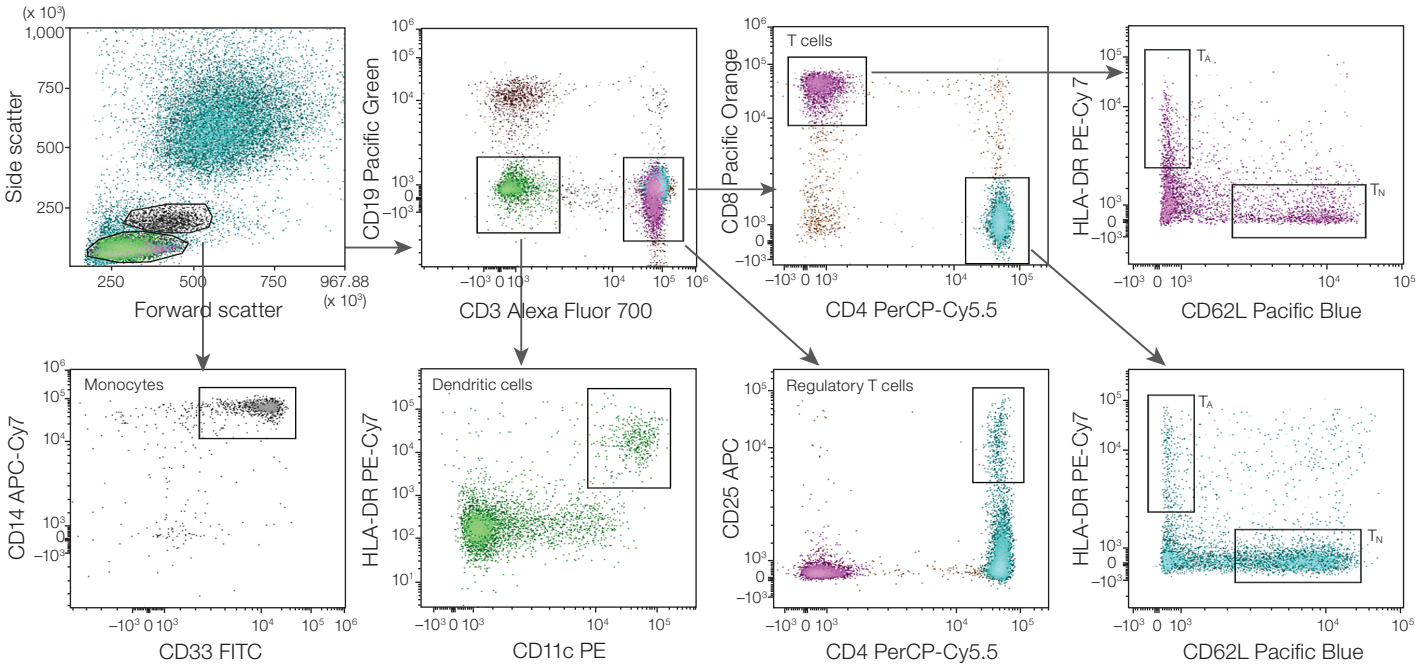


Figure 5. Invitrogen antibodies easily fit into immunophenotype assays. Ten-parameter immunopheotyping of human PBMCs with the Attune NxT Flow Cytometer. Lymphocytes and monocytes were gated based on forward and side scatter profiles. Within the lymphocyte gate, T cells can be isolated based on their expression of CD3 and further subdivided into CD4 and CD8 subpopulations. In addition, regulatory T cells (mediators of dominant peripheral tolerance) express CD4 and CD25. CD62L identifies naive (T_N) $CD4^+$ and $CD8^+$ T cells, whereas HLA-DR is expressed by activated T cells (T_A). Conventional dendritic cells found in peripheral blood are generally negative for T and B cell lineage markers and co-express the integrin CD11c and HLA-DR. Monocytes fall just above lymphocytes based on the scatter profile and express both CD14 and CD33.

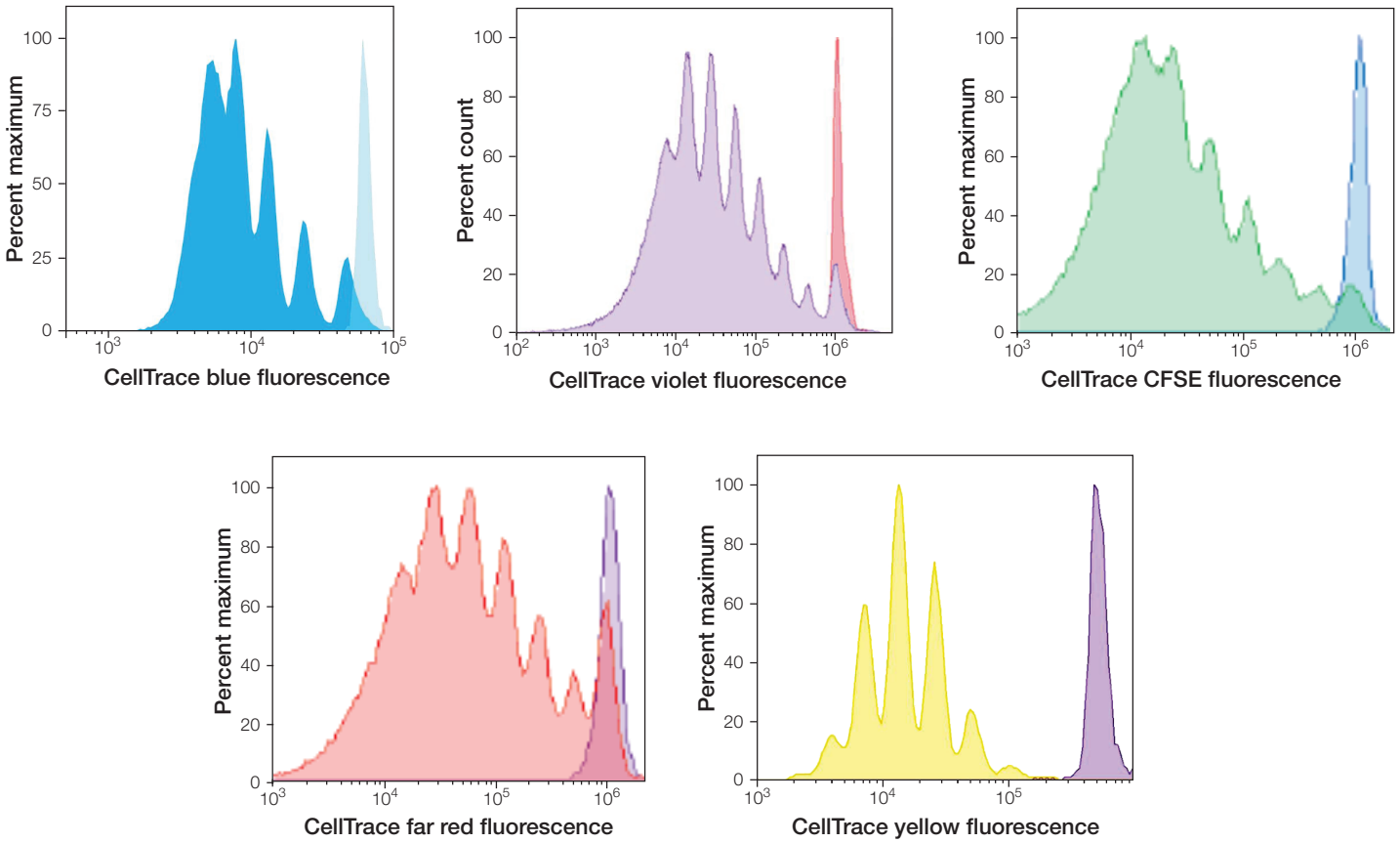


Figure 6. Cell labeling with Invitrogen™ reagents for proliferation of harvested human T lymphocytes. Samples were stained with **(A)** CellTrace violet reagent, **(B)** CellTrace CFSE reagent, **(C)** CellTrace yellow reagent, or **(D)** CellTrace far red reagent. An overlay of the unstimulated parent generation is indicated as the brightest peak on the far right side of each histogram. Cells were traced for seven generations.

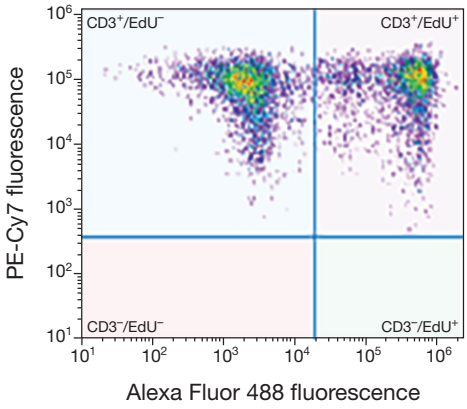


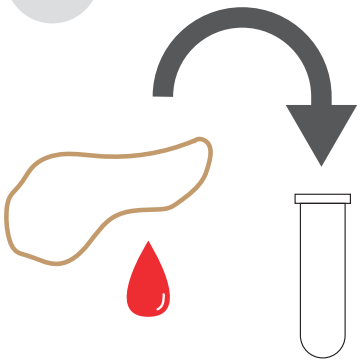
Figure 7. Dual-parameter plot of human T cells assayed for CD3 and DNA strand breaks. CD3 was detected using an anti-CD3 antibody labeled with PE-Cy®7. DNA strand breaks were detected using the Invitrogen™ Click-iT™ Plus EdU Alexa Fluor™ 488 Flow Cytometry Assay Kit.

Analyze whole blood samples

Application spotlight

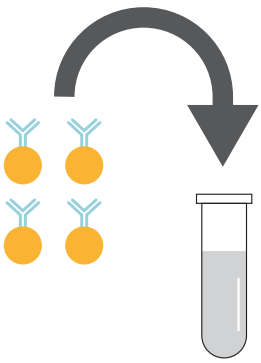
Simplify the workflow with a “no-wash, no-lyse” protocol

1



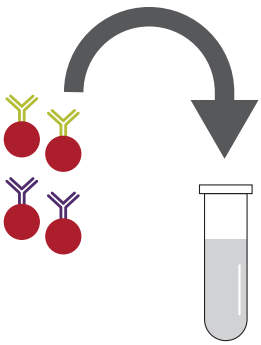
Acquire blood or digest tissue

2



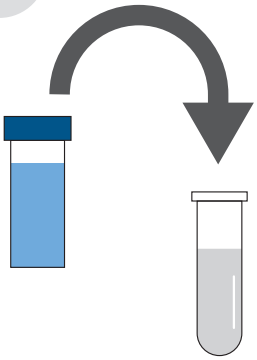
Add live/dead stain

3



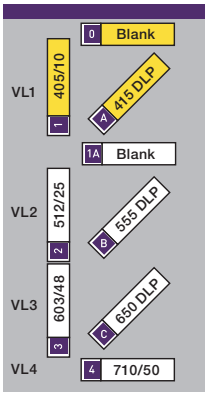
Add labeled antibody to whole blood sample

4



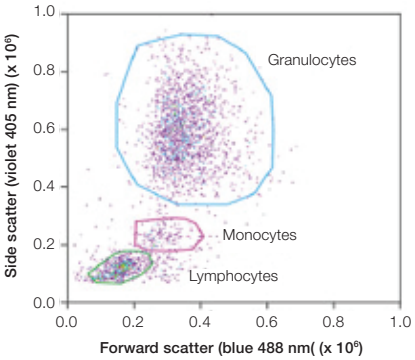
Dilute

5



Change filter configuration to add additional side scatter off the violet laser

6



Run samples on the Attune NxT Flow Cytometer, gate for cell populations

Whole blood analysis with “no-wash, no-lyse” protocol.

Cell imaging

Multicolor cellular imaging gives significant information into the cells and the biological systems being studied. In addition to various protein levels, imaging also gives spatial recognition information and other cellular readouts. One such readout is hypoxia. Cellular responses to reduced oxygen (hypoxic conditions) have been linked to a wide range of human pathologies, including tumor development, atherosclerosis, inflammation, and abnormal angiogenesis. Although the importance of hypoxia in inducing these conditions is well known, creating model systems to accurately control the hypoxic conditions is extremely difficult for most researchers. Until recently, to do this effectively, access to elaborate imaging systems that allow maintenance and precise control of temperature, humidity, and gases (CO_2 and O_2) during an experiment was needed. The Invitrogen™ EVOS™ FL Auto Imaging System with Onstage Incubator provides a solution to this situation. This environmental chamber allows for the precise control of oxygen levels, thereby delivering an effective system for researchers to evaluate cellular responses to hypoxia by long-term fluorescence live-cell imaging (Figure 8).

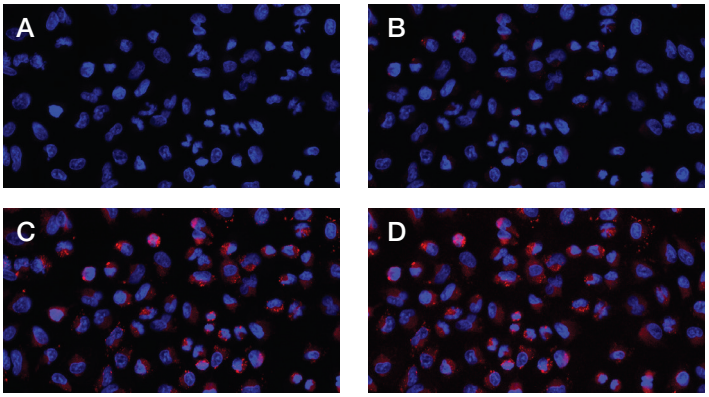


Figure 8. A549 cells stained with Invitrogen™ Image-iT™ Hypoxia Reagent and exposed to different oxygen levels. (A) 20% O_2 **(B)** 5% O_2 **(C)** 2.5% O_2 **(D)** 1% O_2

EVOS FL Imaging System

The EVOS FL Auto Imaging System with Onstage Incubator is designed to eliminate the complexities of long-term live-cell imaging, allowing researchers to focus on data generation, and not instrument operation and maintenance. It allows for time-lapse cell imaging for long-term monitoring of cell cultures. This flexible, high-performance, and affordable solution for live-cell imaging allows you to:

- **Control parameters**—easily control environmental and image acquisition parameters
- **See more**—create time-lapse images of every well of a 96-well plate
- **Save space**—conserve valuable lab space with a small footprint and sleek design
- **Save money**—economical system helps save money with low-cost ownership and operation

Learn more at **thermofisher.com/evos**

High-content imaging for immuno-oncology

Eliminate the complexities for cellular analysis

High-content analysis (HCA) provides both:

- Single-cell resolution for the quantitative measurements using multiple fluorescence channels
- Visualization of each cell with spatial resolution (Figure 9)

Use cell-based imaging screens to identify mechanisms of actions critical for immuno-oncology research. Important applications of the technology include understanding cell-signaling pathways and cellular toxicity. Cell-based assays are increasingly being used to monitor responses by providing a reflection of cell complexities in addition to traditional biochemical assays.

Benefits of cell-based imaging screens include:

- 7-channel LED excitation for a wider probe selection
- Use of wide-field, bright-field, and confocal modes in the same assay
- Compatibility with microscope slides to 1,536-well plates
- High-sensitivity detection and laser-based autofocus helping to reduce photobleaching and toxicity
- Intelligent software enabling faster scan times
- Data analysis during image acquisition for fastest time to results

Reduce assay time and capture more detail. In this example, the Thermo Scientific™ CellInsight™ CX7 HCA Platform is used to detect cellular efficacy and off-target side effects of different chimeric antigen receptor (CAR) T cell therapies (Figure 10).

Figure 9. CX7 High Content Analysis Platform. The is the only high-content screening (HCS) platform to incorporate 4-color, bright-field, 7-color fluorescence, and confocal imaging. The system provides the flexibility and robustness necessary for all HCS workflows and is compatible with the existing informatics infrastructure.

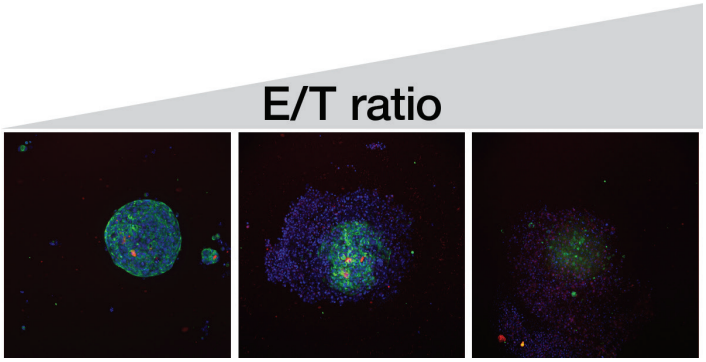


Figure 10. CAR T invasion into cancer spheroids. HCC827 spheroids formed using spheroid microplate for 48 hours. Twenty-four hours after EGFR scFv-CD28-CD3ε CAR T cell addition (ProMab Biotechnologies), spheroids were stained for cytokeratin-7 (green) and CD3ε (red), with Hoescht nuclei counterstain (blue). As effector-to-target (E/T) ratio is increased from 10:1 to 40:1, invasion of the CAR T cells into the HCC827 tumor spheroid and subsequent tumor cell lysis is visible. Images courtesy of Corning Inc., obtained using CellInsight CX7 HCA Platform in confocal mode using 10x objective.

Imaging tumors

Application spotlight

Quickly scan your solid tumor samples

1

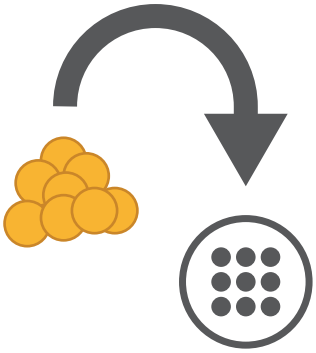
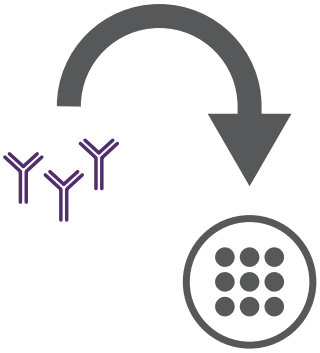


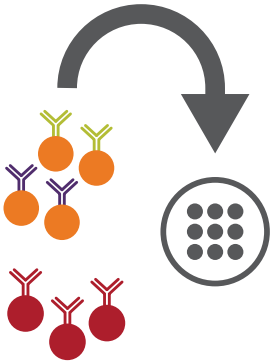
Plate cells

2



Treat cells with antibodies, small molecules, siRNA, or CRISPR

3



Add label or dye

4

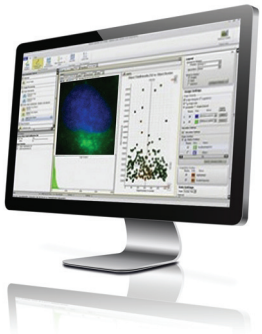
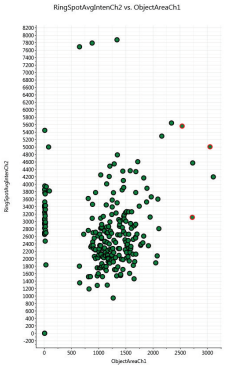


Image acquisition and object identification (segmentation)

5

Scan plate

6



Data visualization and analysis

High-throughput imaging using the CellInsight CX7 HCA Platform for CAR T cell engineering.

Cytokine profiling with immunoassays

Application spotlight

Save time and optimize

Cytokine profiling of samples is an important part in immuno-oncology research as it is used to monitor for both cytokine release syndrome (CRS) and the effectiveness of the CAR T cell therapy itself. A great deal is still currently unknown about the cytokines that are released as well as their origins. The monitoring of CRS is the most common off-target severe adverse event of immuno-oncology therapy.

Detection and quantitation of protein analytes from various biological samples indicate a multitude of biological and pathological events. ELISAs, Luminex® multiplex platforms, and new-generation immunoassays are routinely used for quantitative assessment of soluble proteins such as cytokines, chemokines, growth factors, and other immunological markers.

Invitrogen™ immunoassays using Luminex® xMAP® technology enable the simultaneous quantitation of up to 80 different analytes using only 25–50 µL of sample.

As immunological and biological systems comprises networks of secreted proteins, including cytokines, chemokines, growth factors, and other proteins, multiplex immunoassays are an efficient and time-saving method for biomarker profiling of a large set of proteins from a small sample. As such, multiplex immunoassays have proven to be an invaluable tool for the comprehensive study of biological systems.

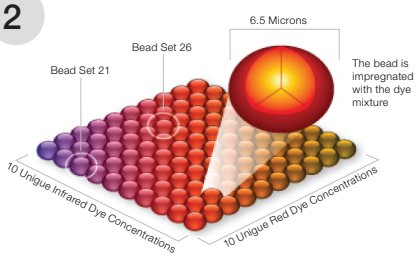
- **Fast**—quantitate proteins in 3 hours with little hands-on time
- **Quantitative**—quantitate up to 80 proteins simultaneously
- **Easy**—as easy to run as an ELISA
- **Expand**—expand or change your biomarker profile as your research advances

To learn more about our immunoassay offerings, including Invitrogen™ ProcartaPlex™ multiplex products and services, go to **thermofisher.com/immunoassays**

1

Use small amount of blood or serum

2



Capture and label protein on ProcartaPlex panels

3

Analyze results on the Luminex® instrument; choose from FLEXMAP 3D® (shown above), MAGPIX®, or the Luminex® 200™ system

Multiplex immunoassay application spotlight for protein quantitation.

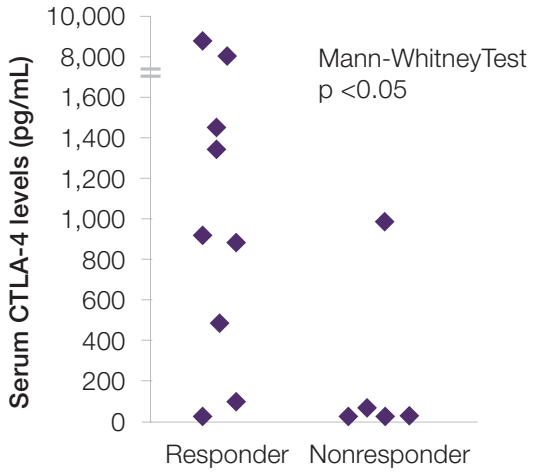


Figure 11. sCTLA-4 levels were measured by ELISA and individual values plotted according to clinical responses to the monoclonal antibody ipilimumab.

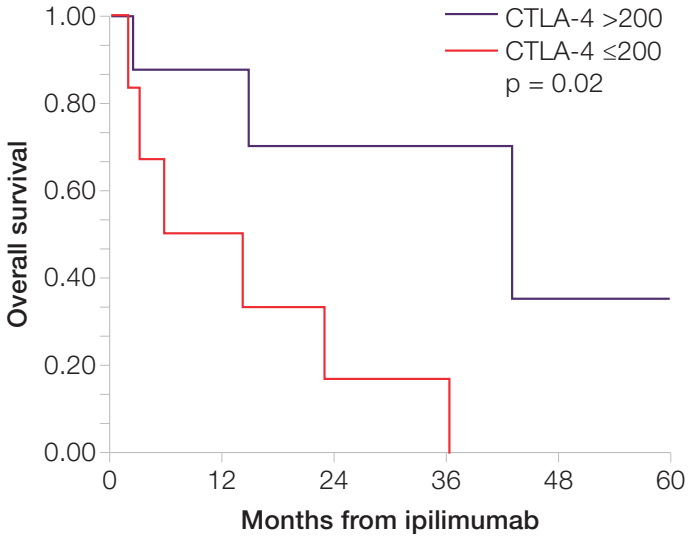


Figure 12. Overall survival (5 years) of patients treated with ipilimumab—comparing those with >200 pg/mL serum sCTLA-4 to those with ≤200 pg/mL.

Biomarker measurement using ELISA kits

Speed, sensitivity, reliability, and compatibility with standard clinical laboratory equipment make immunoassays such as Invitrogen™ coated ELISA kits the method of choice for biomarker evaluation.

Quantification of soluble CTLA-4 (sCTLA-4) levels by ELISA could be a forthcoming method to distinguish ipilimumab responders from non-responders since elevated sCTLA-4 serum levels correlate with clinical benefit to ipilimumab (Figure 11), and patients with elevated sCTLA-4 also show significant survival benefit over those with low sCTLA-4 levels (Figure 12) [16].

Cell Therapy Systems (CTS) products

Achieve a seamless transition from bench to clinic with high-quality reagents

Cell engineering solutions

We offer complete cell engineering solutions to meet your gene editing and cell delivery needs, including transfection reagents, lentiviral transduction, electroporation, and award-winning gene editing tools and solutions.

- Lentiviral production
  Whether you are using adherent or suspension vector production, we can help you achieve high-titer and cost-effective lentiviral vector production.
  - Suspension lentiviral production at any scale—Our latest innovation, the Gibco™ LV-MAX™ Lentiviral Production System, is the first complete lentiviral production system for suspension cell cultures, and it is optimized to allow you to seamlessly and efficiently scale your lentiviral production with high titers in a serum-free system.

  - Advanced lipid nanoparticle technology for superior lentiviral production in adherent cultures—Invitrogen™ Lipofectamine™ 3000 Transfection Reagent is a highly efficient, cost-effective tool for lentiviral production. This versatile reagent enables high viral titers even with genes that are large or difficult to package.

To learn more about our lentiviral solutions, go to **thermofisher.com/lentiviral**

CAR T cell therapy media and reagents

In addition to our characterization tools, we have capabilities that span the immunotherapy workflow from isolation to gene editing and cell expansion. Our products, services, and support are designed to facilitate a seamless transition from research to commercialization, with a goal to reduce the time from your initial discovery to an approved therapy.

Gibco™ CTS media and reagents are manufactured in accordance with cGMP for medical devices, 21 CFR Part 820, and are designed to help you translate your cell therapy to clinical applications with extensive safety testing and traceability documentation to facilitate regulatory approval, so you can transition your cell therapy to the clinic with confidence.

- **Cell isolation and activation**–Gibco™ CTS™ Dynabeads™ CD3/CD28 magnetic beads provide a trusted technology platform for *ex vivo* T cell isolation, activation, and expansion for immunotherapy [17-19].

- **Cell expansion**–Gibco™ cell culture represents over 50 years of applying deep scientific cell culture expertise. Used in clinical cell therapy applications, our broad portfolio of CTS media and reagents has been developed to support your transition to the clinic.

  - Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM)—complete xeno-free formulation proven for clinical success and specifically developed for the growth and expansion of human T lymphocytes.
  - Gibco™ CTS™ Immune Cell Serum Replacement—a defined xeno-free formulation proven for clinical use and designed to support expansion of in vitro–cultured human T cells when added as a supplement to a basal cell culture medium such as CTS OpTmizer T Cell Expansion SFM or Gibco™ AIM V™ Medium.

To learn more about our CAR T cell therapy media and reagents, go to **thermofisher.com/ctsimmunotherapy**

The ability to learn more about cancer, develop powerful therapeutics, and bring it to market is what drives our immuno-oncology research. Gibco™ CTS™ products can partner with your group or company to develop precision therapeutics from cell culture to production (Figure 13).

Develop precision therapeutics with CTS™ Dynabeads™ magnetic beads and DynaMag™ magnets. This gentle and efficient technology provides a trusted technology platform from which the beads can be used to isolate T cells and provide both the primary and co-stimulatory signals required for activation and expansion. Applications include cell expansion for CAR T and T cell receptor (TCR) cells.

When you choose CTS products, you can expect:

cGMP-compliant manufacturing

- Manufactured in conformity with cGMP for medical devices, 21 CFR Part 820 of the regulation
- FDA-registered manufacturing site with an ISO 13485–certified quality management system

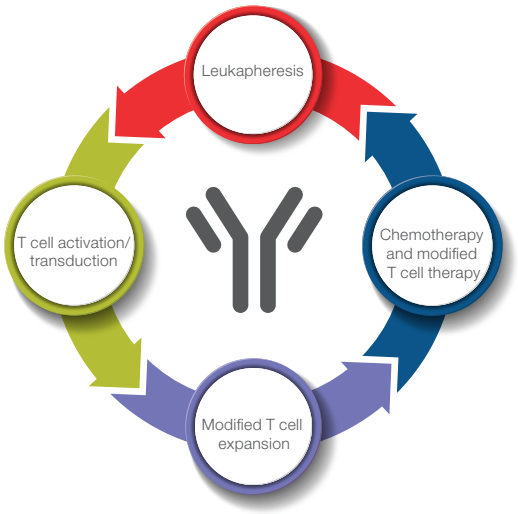
Seamless transition to the clinic

- Traceability documentation, including certificates of analysis, certificates of origin, and drug master files
- Extensive QC testing for sterility, endotoxin, adventitious agent, and mycoplasma on most products

Expert support

- Experienced professionals to help navigate regulatory processes from research to commercial phase
- Cell therapy experts to help answer your questions

Figure 13. Gibco CTS research to clinical research benefits.



CAR T cell therapy workflow—from leukapheresis to CAR T manufacturing to patient infusion.

CTS cell expansion benefits include:

- Isolated and activated T cells enable efficient gene transduction
- Expanded T cells have a central memory T cell phenotype with persistence *in vivo* expansion of 100–1,000 fold in 9–14 days
- Scalable, effective separation of cells and removal of beads after expansion

Indications and product information

- Available for use in clinical trials under an approved investigational new drug (IND) application
- For research use or non-commercial manufacturing of cell-based products for clinical research
- Manufactured in conformity with cGMP; the site is FDA-registered and has an ISO13485–certified facility

To learn more, go to **thermofisher.com/us/en/home/clinical/clinical-translational-research/cell-therapy.html**

Solutions for the immunotherapy workflow

From research to the clinic and beyond, we have solutions to help you achieve your cell therapy goals

Cell isolation and activation

Products intended for *ex vivo* isolation, activation, and expansion of human T cells in translational research with best-in-class purity, yield, and function.

Cell engineering

Complete cell engineering solutions to meet your T cell delivery needs, including vector construction, production and purification, vector delivery, electroporation, and gene editing tools.

Cell expansion

Extensive selection of cell expansion media and reagents, including serum-free media, serum replacement, growth factors, wash buffers, and cryopreservation solutions. We also offer bioreactors, cultureware, cell culture bags, and custom media for a complete cell expansion solution.

Cell analysis

Equipment, tools, and reagents for cell counting, whole-cell analysis, protein analysis, and genetic analysis, along with safety testing, including endotoxin and mycoplasma. State-of-the-art cell characterization tools for your in-process and lot-release testing needs.

Cell delivery

Clinical trial logistics support, cGMP-compliant global biobanking, cryogenic distribution expertise, and comprehensive supply chain management for autologous and allogeneic cell therapies.

Service and support

We are committed to helping progress research of immuno-oncology cell pathway regulators, genetically modified T cells, and other immunity-based cell therapy strategies. Flow cytometry products, from sample preparation to executing experiments, are designed to facilitate research and development of advanced immunotherapy.

To learn more, go to **thermofisher.com/flowcytometry**

Resources

- **Flow Cytometry Support Center**
- **Flow Cytometry Resource Center**
- **Flow Cytometry Learning Center**
- **T Cell Proliferation and Stimulation eLearning Course**
- **Antibody and Immunoassay Resource Center**
- **Fluorescence SpectraViewer**
- **High-Content Analysis Reagents**
- **Flow Cytometry Protocols**
- **Molecular Probes™ Handbook**
- **High-Content Applications and Protocols**
- **Recommendations to build multicolor panels**
- **Resources to create high-content imaging**
- **Immunofluorescence Selection Guide**

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