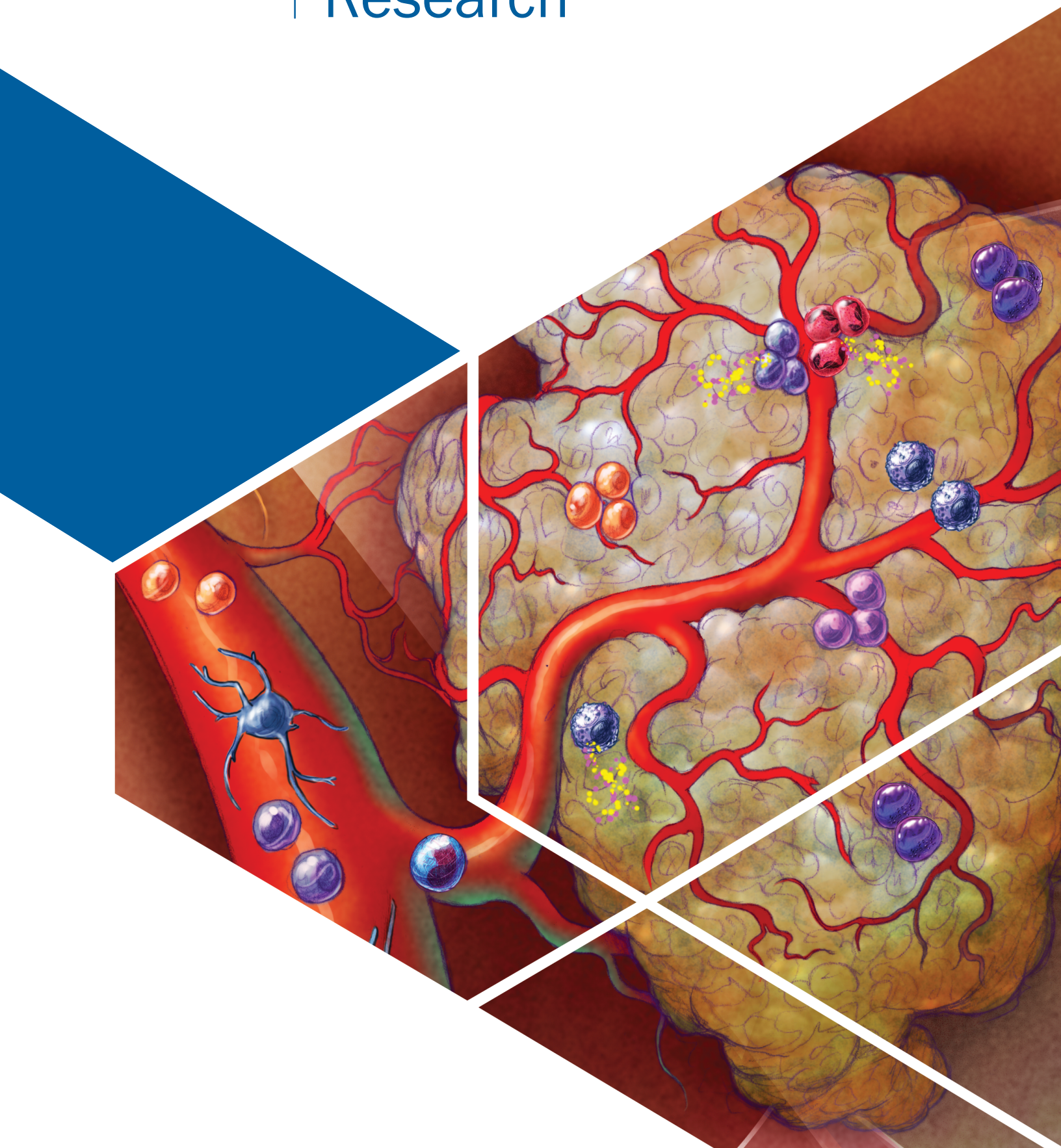
R&D SYSTEMS
a bio-techne brand

Cancer Immunotherapy Research



Cancer Immunotherapy Research

Cancer immunotherapy has become an extremely promising approach for the treatment of cancer. Immune checkpoint blockade, adoptive cell transfer, and altering the tumor microenvironment are three approaches to cancer immunotherapy that are being actively investigated. More research in all of these areas is needed to gain a better understanding of responses to cancer immunotherapy and to direct more effective therapeutic strategies moving forward. The Bio-Techne life science brands – R&D Systems, Tocris Bioscience, Novus Biologicals, and ProteinSimple – offer an extensive selection of reagents, kits, small molecules, assay platforms, and custom services that can facilitate your research and assist in driving this important field forward.

Adoptive T Cell Therapy

- *In vitro* T cell activation and expansion
- T cell characterization
- T cell isolation
- *In vitro* T cell function

Tumor Microenvironment & Inhibitory Receptor Blockade

- Investigate the tumor microenvironment
- Block immune checkpoints
- Monitor T cell exhaustion

Custom Reagents & Contract Services

- Leverage Bio-Techne's legacy of scientific innovation to advance your research

Adoptive T Cell Therapy

Adoptive cell therapy consists of generating large numbers of anti-tumor lymphocytes – tumor-infiltrating lymphocytes (TILs), transgenic TCR T cells, chimeric antigen receptor (CAR) T cells, etc. – and injecting them into patients. Regardless of the specific strategy being utilized, cells need to be activated, expanded, characterized, isolated, and functionally verified. New CAR strategies, continued improvements to T cell expansion methods, and combining adoptive cell transfer with other approaches such as immune checkpoint blockade, will be crucial moving forward. Trust our wide range of proteins, antibodies, kits, and assays to facilitate your adoptive T cell therapy research.

In Vitro T Cell Activation and Expansion

Adoptive cell therapy experiments require the expansion and stimulation of T cell populations to achieve the high numbers needed for therapy. The process of expanding T cells to therapeutic numbers is time consuming and costly. In order for adoptive cell transfer to be a viable option moving forward more research is needed to improve the efficiency of current T cell expansion methods. Our hope is that the high-quality proteins and antibodies we offer, and the expertise behind their development, can help move the field of adoptive cell therapy forward.

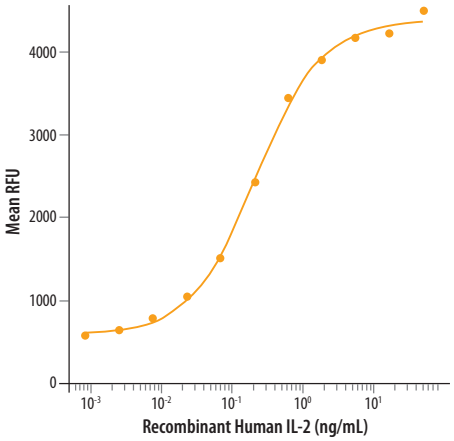
Activate T Cells with Anti-CD3/OKT3 and Anti-CD28 Antibodies

- Our antibodies provide consistent performance and are 100% guaranteed to work in the application and species listed.

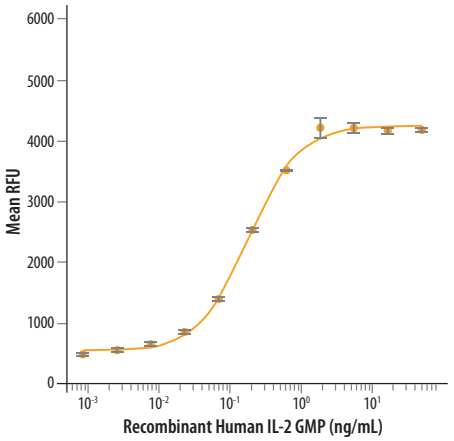
Expand Cells with Our Recombinant IL-2 Proteins

Our industry-leading proteins are the ideal choice for consistent and reliable results.
Features of our IL-2 proteins include:

- Highly cited
- Lowest endotoxin specification (<0.1 EU/µg) on the market
- Bioactivity demonstrated with relevant bioassay data
- GMP-grade available
- ProDots® Protein available



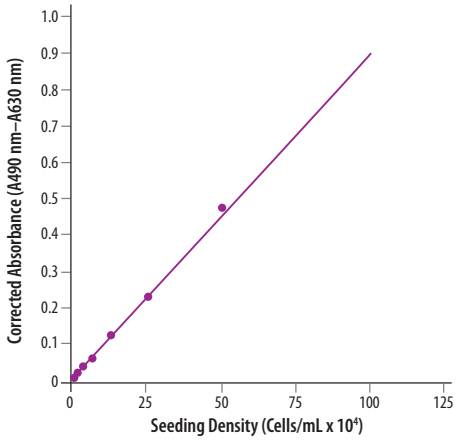
Recombinant Human IL-2 (R&D Systems; Catalog # 202-IL) Stimulates Cell Proliferation of T Cells



GMP-grade Recombinant Human IL-2 (R&D Systems; Catalog # 202-GMP) Stimulates Cell Proliferation of T Cells

Measure Cell Proliferation

The TACS® XTT Cell Proliferation Assay avoids radioactivity and gives reproducible and sensitive results. XTT is added directly to the culture medium and cleavage of the tetrazolium salt to formazan occurs only in metabolically active cells.



Relative Cell Number Measured with the TACS® XTT Cell Proliferation Assay (R&D Systems; Catalog # 4891-025-K)

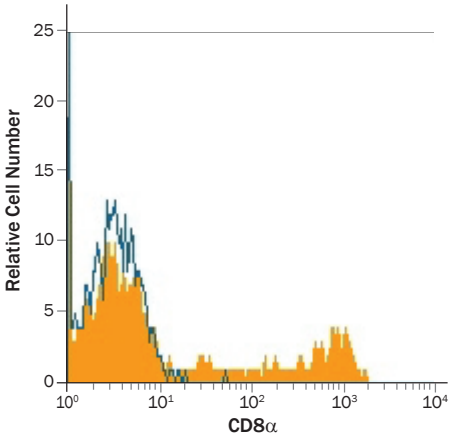
T Cell Characterization

Flow Cytometry Antibodies for T Cell Markers

The table below lists markers commonly used to characterize T cell subsets, T cell activation, memory T cells, and regulatory T cells. Visit rndsystems.com to find the antibodies you need for your flow cytometry panel.

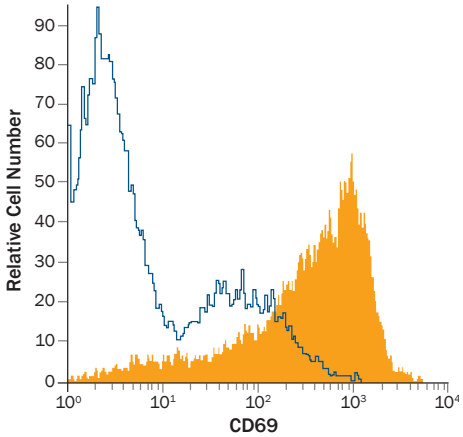
T Cell Subsets	T Cell Activation	Memory T Cells	Regulatory T Cells
CD3	CD25	CD45RO	CD25
CD4	CD38	CD27	FoxP3
CD8	CD69		CD127
	HLA-DR		

Choose from 29 CD8 Flow Cytometry Antibodies



Detection of CD8α on Human Lymphocytes with an anti-Human CD8α Alexa Fluor®488-conjugated Antibody (R&D Systems; Catalog # FAB1509G)

Monitor T Cell Activation



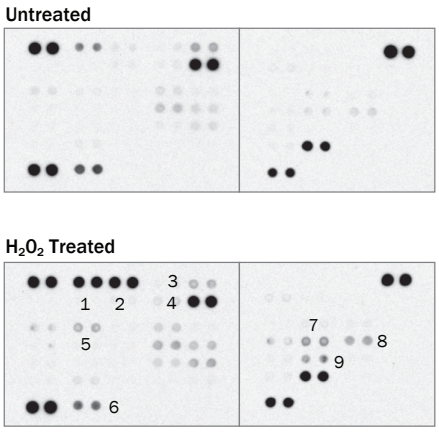
Detection of CD69 on Human Lymphocytes with an anti-Human PE-conjugated Antibody (R&D Systems; Catalog # FAB23591P)

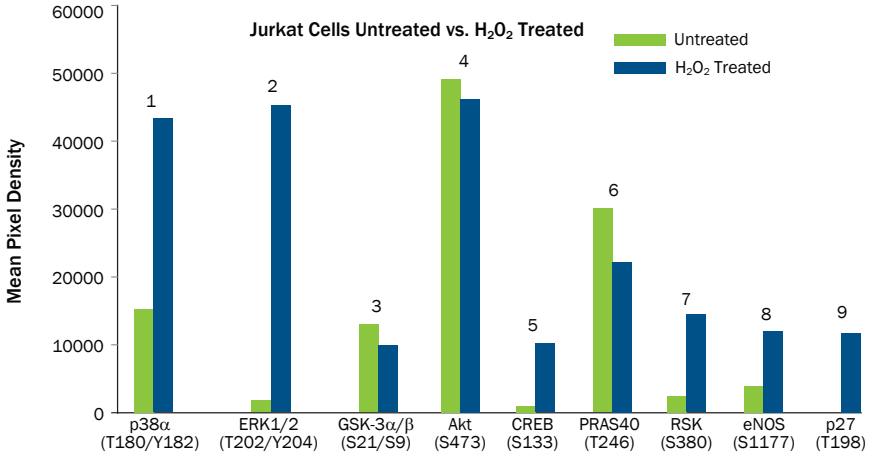
Learn more and find products | rndsystems.com/markers

Analysis of CAR Signaling: Proteome Profiler™ Antibody Arrays

Chimeric antigen receptor (CAR) T cell transfer has had success as a treatment for leukemia and lymphoma, but solid tumors have been more challenging due to the rarity of true tumor-specific target molecules and the immunosuppressive nature of the tumor microenvironment. A better understanding of CAR signaling pathways and how they differ from T cell receptor (TCR) signaling pathways is needed to better inform future CAR-based strategies targeting solid tumors. What aspects of endogenous TCR signaling are missing? Is the cytokine secretion profile different between CAR T cells and conventional T cells? Proteome Profiler™ Antibody Arrays are ideally suited to address questions like these by providing an unbiased analysis of both intracellular and extracellular T cell responses.

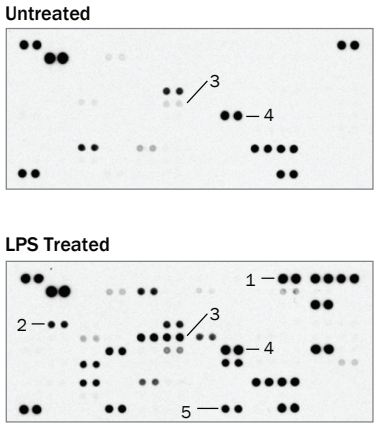
Simultaneously Assess the Phosphorylation Status of 43 Kinases

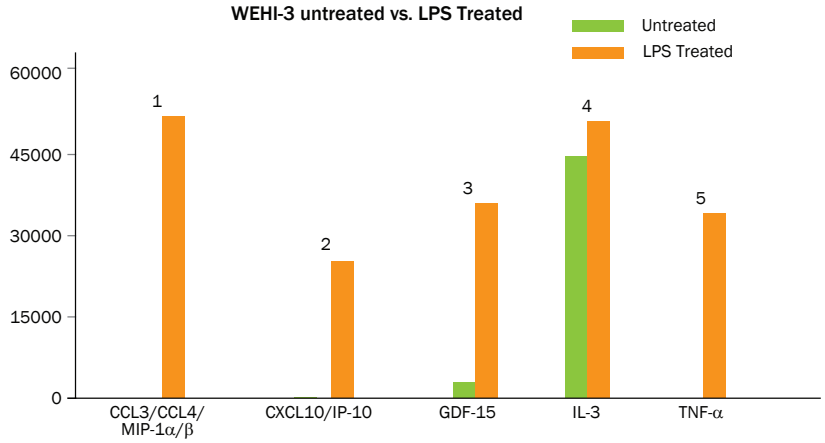




Signal Transduction Profile of Leukemia Cells Measured with the Proteome Profiler™ Human Phospho-Kinase Array Kit (R&D Systems; Catalog # ARY003B)

Simultaneously Monitor the Expression of 111 Cytokines





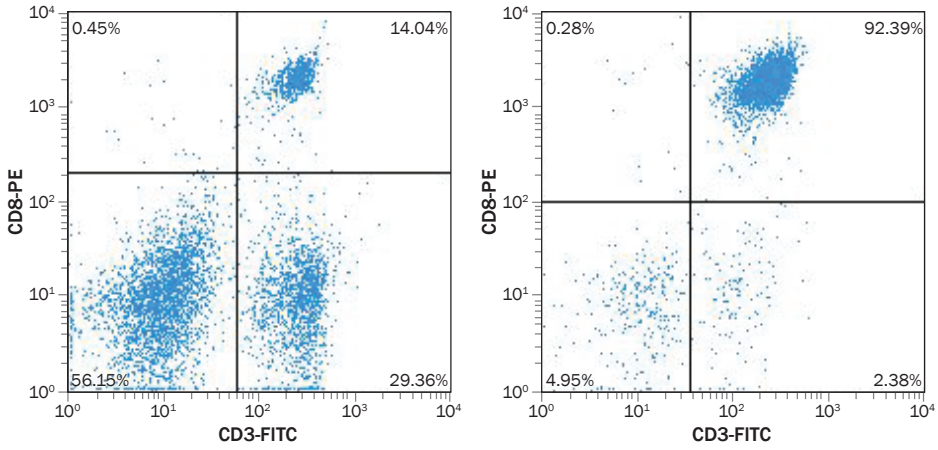
Cytokine Secretion Profile of Leukemia Cells Measured with the Proteome Profiler™ Mouse XL Cytokine Array Kit (R&D Systems; Catalog # ARY028)

Learn more and find products | rndsystems.com/car

T Cell Isolation

Isolate the cells you need for your adoptive cell transfer experiments with our MagCellect™ Cell Selection Kits. Using these kits, unwanted cells are magnetically tagged with a biotinylated antibody cocktail and streptavidin ferrofluid. The cell suspension is subsequently placed in a magnetic field, and the desired, untouched cell population of choice is harvested by aspiration and immediately available for downstream applications.

Isolate CD8+ T Cells

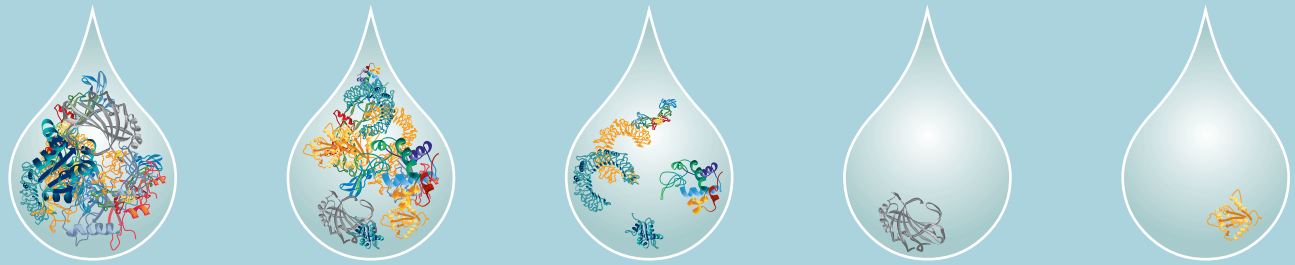


Enrichment of Mouse Splenocytes using the MagCellect™ Mouse CD8+ T Cell Isolation Kit (R&D Systems; Catalog # MAGM203)

Learn more and find products | rndsystems.com/cellisolation

Choose the Right Immunoassay to Get Your Answers!

Whether you need qualitative or quantitative results, have limited or abundant sample, require single or multi-analyte analysis, you will be able to find an optimal solution at R&D Systems.



Proteome Profiler™ Arrays	Luminex® Assays	Simple Plex™ Assays	DuoSet® ELISAs	Quantikine® ELISAs
No specialized equipment required, affordable screening tool.	Quantitative multiplex for up to 100 analytes, small sample size.	Multi-analyte parallel immunoassay, small sample size, easy to use, and sensitive.	Flexible, economical solution for single analyte ELISA, over 1,00 available.	The industry gold standard ELISA to confirm results. Over 450 available.

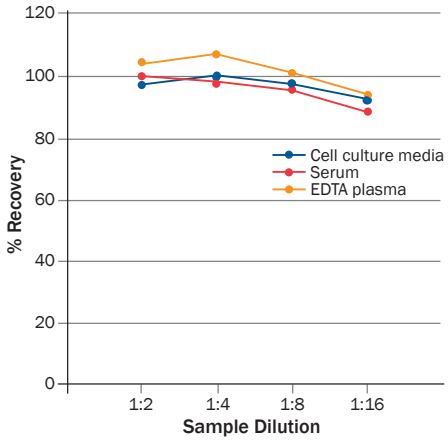
Learn more and find products | rndsystems.com/immunoassays

In Vitro T Cell Function

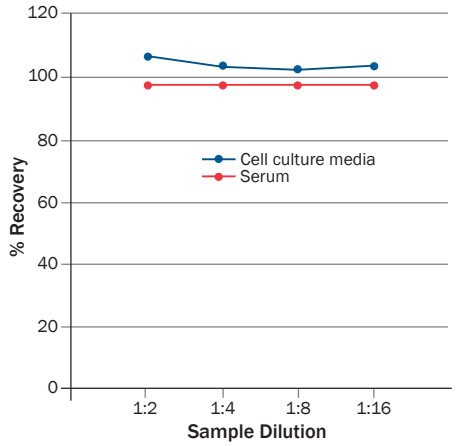
Measure IFN-γ Protein Levels

Trust the most-referenced ELISAs on the market to verify T cell function by measuring IFN-γ secretion. Our Quantikine® ELISA kits are the industry gold standard and have been exhaustively tested for superior quality and reproducibility.

Measure Human and Mouse IFN-γ Levels with Quantikine® ELISAs



Linearity of the Human IFN-γ Quantikine® ELISA Kit (Catalog # DIF50)



Linearity of the Mouse IFN-γ Quantikine® ELISA Kit (Catalog # MIF00)

Simple Plex™ Assays

The Ella™ platform runs Simple Plex™ Assays. These novel assays are fully automated, quantitative, single or multianalyte immunoassays that will transform your research possibilities:

- Quantify up to 4 analytes in a single 25 µL sample. Ella™ is sensitive enough to detect sub-picogram levels of protein and has a 4-5 log dynamic range to help avoid sample dilution.
- Setup only takes 5 minutes. You simply pipette your samples into our cartridge, which automates the entire assay for you. Data is ready in 60 minutes.
- Over 140 human analytes are currently available. And with our catalog being powered by R&D Systems, our menu is unlimited. Just let us know what you need!

Measure Cytotoxicity

The Calcein AM Cell Viability Kit (R&D Systems; Catalog # 4892-010-K) provides a simple, rapid, and accurate method to measure cell viability and/or cytotoxicity. Calcein AM is a non-fluorescent, hydrophobic compound that easily permeates intact, live cells. The hydrolysis of Calcein AM by intracellular esterases produces Calcein, a hydrophilic, strongly fluorescent compound that is well-retained in the cell cytoplasm. Cells grown in black-walled plates (R&D Systems; Catalog # DY991) can be quantified in less than two hours using a fluorescence plate reader with excitation at 490 nm and emission at 520 nm. The kit contains reagents for ~1000 tests.

Tumor Microenvironment & Inhibitory Receptor Blockade

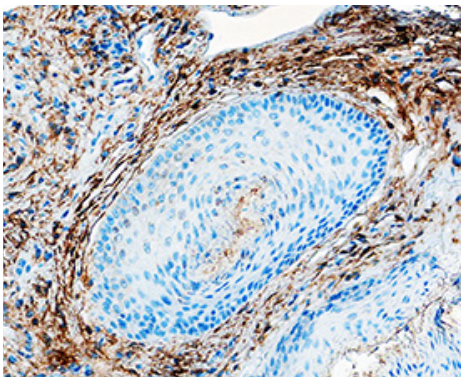
A large body of evidence suggests that tumors create an immunosuppressive microenvironment that inhibits the immune system's natural ability to recognize and destroy tumor cells. This is accomplished via several mechanisms, including the recruitment of immunosuppressive cells, activation of immune checkpoint pathways, and exclusion of T cells. Gaining a better understanding of these immunosuppressive mechanisms will provide an important step towards developing novel strategies to overcome this obstacle to cancer immunotherapies.

Immunosuppressive Cells

Many cell types are thought to contribute to the generation of an immunosuppressive tumor microenvironment including cancer-associated fibroblasts, myeloid-derived suppressor cells, regulatory T cells, and tumor-associated macrophages. Read below for a brief description of mechanisms by which they contribute to immune suppression. We offer proteins, antibodies, ELISAs, assays, and small molecules for your workflow.

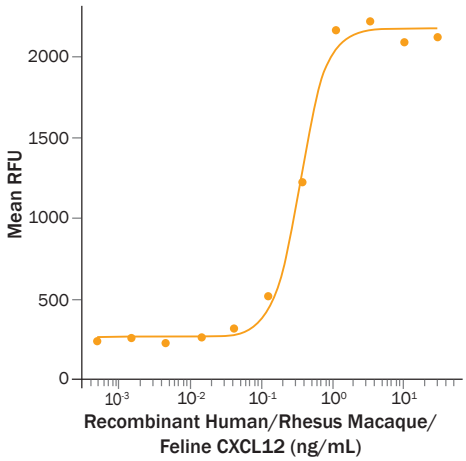
Cancer-associated Fibroblasts (CAFs) restrict T cells to the stroma, preventing them from accumulating in the vicinity of cancer cells. This is accomplished, at least in part, by production of dense extracellular matrix and secretion of CXCL12.

Identify CAFs with FAP Antibodies



Detection of Fibroblast Activation Protein alpha/FAP in human basal cell carcinoma with an anti-Human FAP Antibody (R&D Systems; Catalog # AF3715)

Investigate CAF-mediated T Cell Exclusion with Recombinant Chemokines

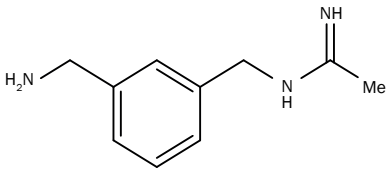


Recombinant Human/Rhesus Macaque/Feline CXCL12 (R&D Systems; Catalog # 350-NS)-mediated chemoattraction of pro-B cells expressing CXCR4

Myeloid-derived Suppressor Cells (MDSCs) contribute to the immunosuppressive tumor microenvironment by producing Arginase 1/ARG1, upregulating iNOS, and secreting immunosuppressive cytokines.

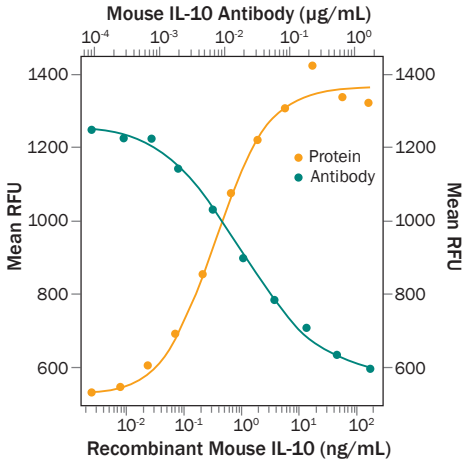
Inhibit iNOS with Small Molecules

TOCRIS
a bio-techne brand



1400W (Tocris; Catalog # 1415) is a potent and selective iNOS inhibitor. The compound is cell permeable and active *in vivo*.

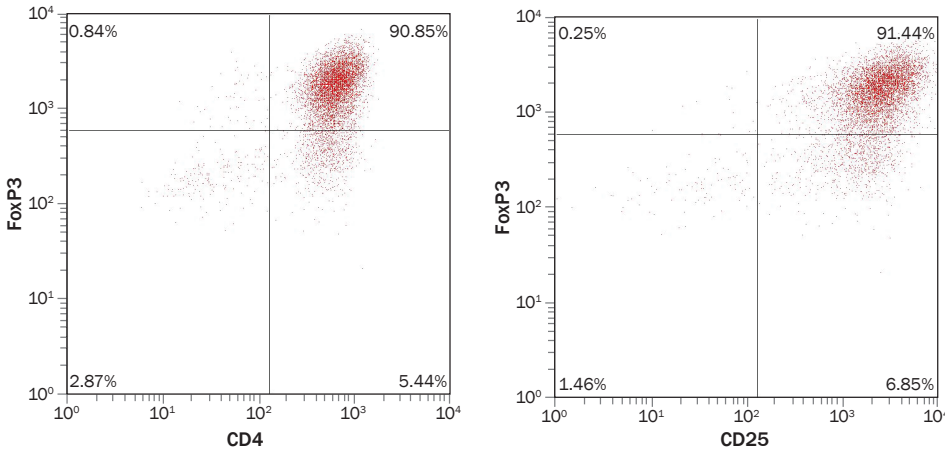
Block MDSC-secreted IL-10 with Neutralizing Antibodies



Neutralization of IL-10-induced cell proliferation with an anti-Mouse IL-10 Antibody (R&D Systems; Catalog # MAB417)

Regulatory T Cells (Tregs) represent a significant suppressive population in tumors and they function by inhibiting the activities of $CD4^+$ and $CD8^+$ effector T cells, natural killer cells, NKT cells, and antigen-presenting cells through secretion of immunosuppressive cytokines and production of cytolytic factors.

Simplify Immune Cell Differentiation with CellXVivo™ Differentiation Kits



Differentiation of $CD4^+$ T Cells into Treg Cells using the CellXVivo™ Human Treg Cell Differentiation Kit (R&D Systems; Catalog # CDK006)

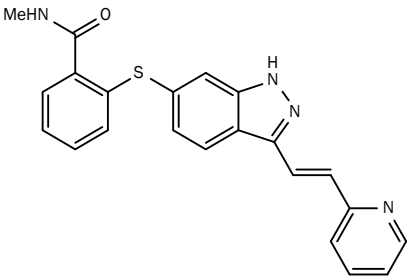
Immune Cell Differentiation at its Simplest

1. **Isolate** your starting immune cell population
2. **Incubate** cells in the Cytokine cocktail from your chosen CellXVivo™ Differentiation Kit.
3. **Verify** and quantify differentiation

Tumor-associated Macrophages (TAMs) induce immune suppression in the tumor microenvironment by mechanisms that are not completely understood, but likely involve the production of TGF-β, VEGF, and CCL18.

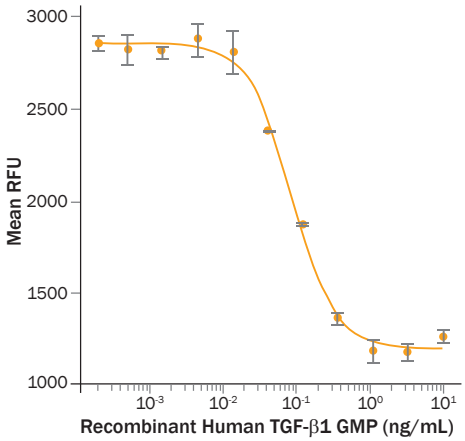
Inhibit VEGF Signaling with Small Molecules

TOCRIS a bio-techne brand



Axitinib (Tocris; Catalog # 4350) is a potent VEGF R1, -2, -3 inhibitor

Investigate TGF-β-mediated Immune Suppression with GMP-grade Proteins



Inhibition of IL-4-induced proliferation with GMP-grade Recombinant Human TGF-β1 (R&D Systems; Catalog # 240-GMP)

Learn more and find products | rndsystems.com/suppressivecells **and** tocris.com

Learn more and find products | rndsystems.com/sisterclones

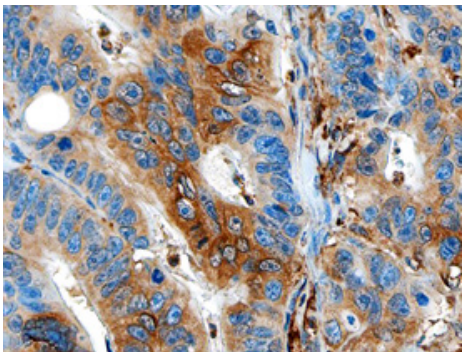
Immune Checkpoint Molecules

Blockade of immune checkpoint molecules, such as CTLA-4 and PD-1/PD-L1, is a promising therapeutic approach for cancer treatment. Unfortunately, blocking CTLA-4 or PD-1/PD-L1 is not universally successful across different cancer types and patients. Co-targeting multiple checkpoint molecules simultaneously could improve the effectiveness of this strategy and is an active area of investigation. Take advantage of the best blocking antibodies on the market to identify the next key immune checkpoint target.

Immune Checkpoint Blockade

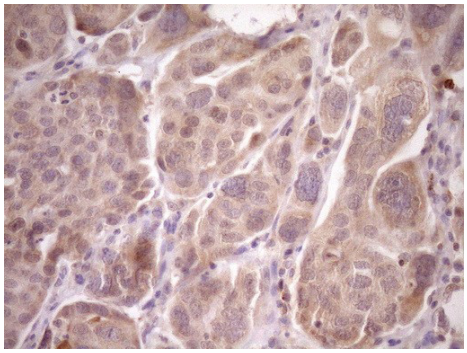
Check for expression of the immune checkpoint molecule of interest and then block its function with our selection of antibodies from R&D Systems and Novus Biologicals.

Monitor the Expression of Immune Checkpoint Molecules



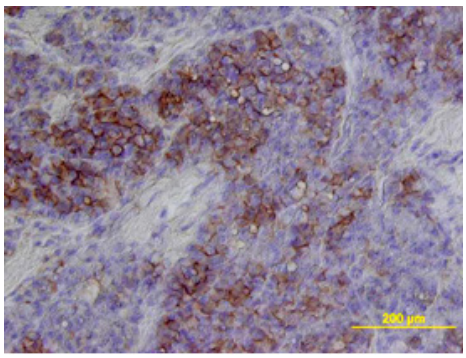
Detection of PD-L1 in Human Colon Cancer with an anti-Human PD-L1 Antibody (R&D Systems; Catalog # MAB1561)

Block PD-L1 Function (R&D Systems; Catalog # AF156)



Detection of LAG-3 in Human Ovarian Cancer with an anti-Human LAG-3 Antibody (Novus Biologicals; Catalog # NBP2-45581)

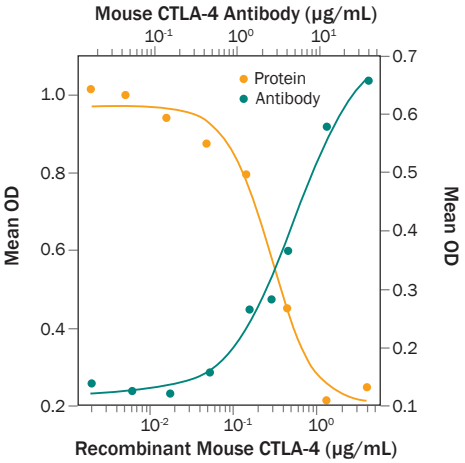
Block LAG-3 Function (Novus Biologicals; Catalog # NBP1-97662)



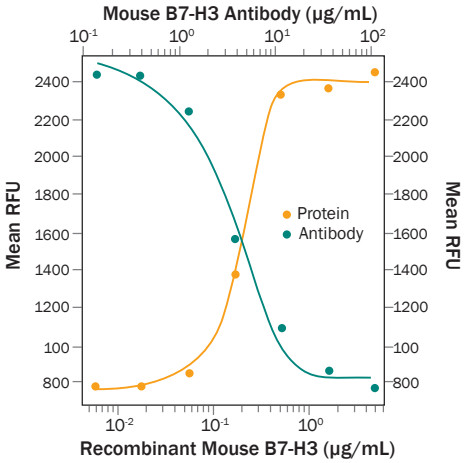
Detection of B7-H3 in Human Melanoma with an anti-Human B7-H3 Antibody (R&D Systems; Catalog # AF1027)

Block B7-H3 Function (R&D Systems; Catalog # AF1397)

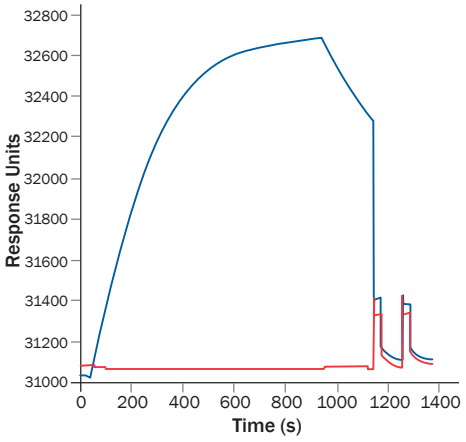
Block the Function of Immune Checkpoint Molecules



CTLA-4 Inhibition of B7-1/CD80-induced IL-2 Secretion and Neutralization by an anti-Mouse CTLA-4 Antibody (R&D Systems; Catalog # MAB434)



Proliferation Induced by B7-H3 and Neutralization by an anti-Mouse B7-H3 Antibody (R&D Systems; Catalog # AF1397)

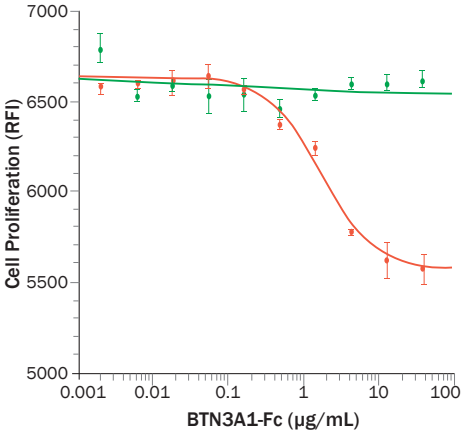


B7-H7 Binding to CD28H is Blocked (red line) by an anti-Human B7-H7 Antibody (R&D Systems; Catalog # MAB80841)

The Widest Selection of Bioactive Immune Checkpoint Proteins Available: New Checkpoint Targets?

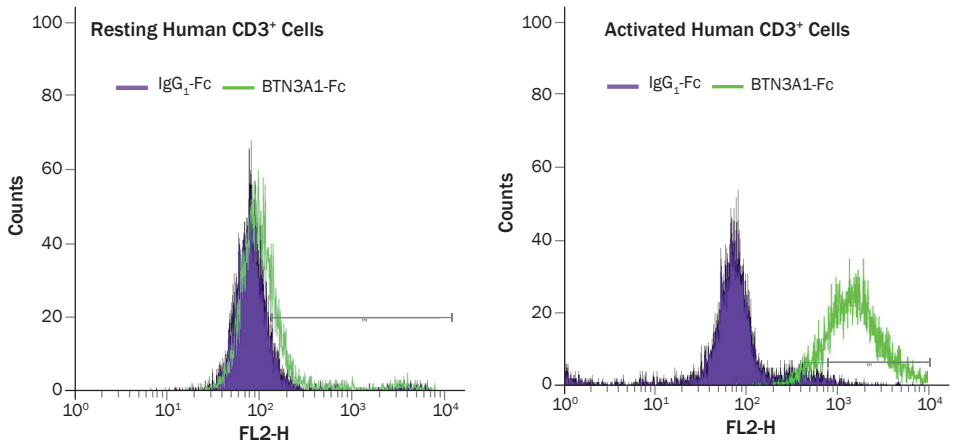
Our bioactive protein families include PD, B7, CD28, TNF, VSIG/TIGIT, Galectins, and more. In addition, less widely studied immune checkpoint proteins are also available and offer promise as future targets for drug discovery. These include Angiopoietin-like ligands and their LILRA/B receptors, and the B7-related Butyrophilin family. Butyrophilins are a novel class of co-stimulatory/co-inhibitory molecules that are structurally related to the B7 family and appear to have similar immunomodulatory functions. These parallels suggest that the butyrophilins or their receptors may also serve as drug targets. Recognizing this potential, R&D Systems exclusively offers bioactive recombinant butyrophilin proteins to further research on these molecules.

Modulate Immune Responses



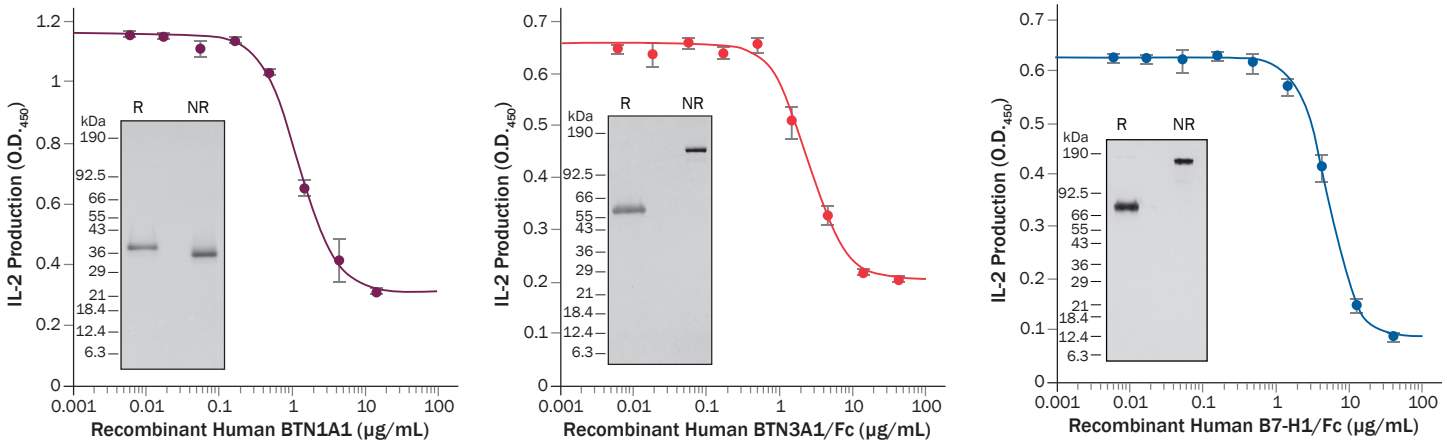
Recombinant Human BTN3A1 (R&D Systems; Catalog # 8539-BT) Inhibits Human T Cell Proliferation (orange line) in a Dose-Dependent Manner

Identify Binding Partners for Butyrophilins



Recombinant Human BTN3A1 (R&D Systems; Catalog # 8539-BT) Binds to Activated Human T Cells

Our Highly Bioactive and Pure Recombinant Butyrophilins are Ideal for Screening Potential New Drugs



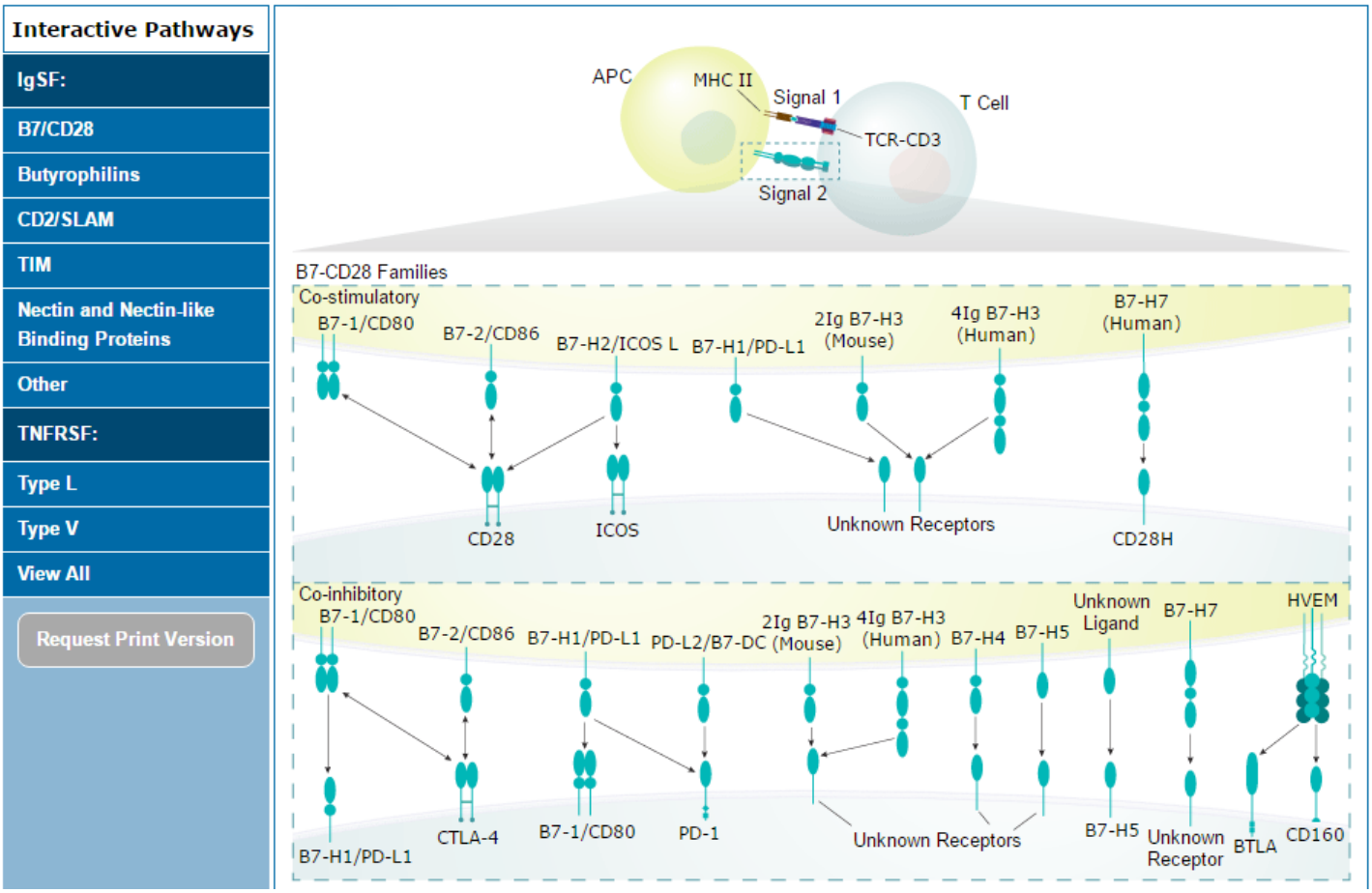
BTN1A1 and BTN3A1 Inhibit Anti-CD3-Induced IL-2 Production by Human T Cells in a Manner Similar to B7-H1/PD-L1

Learn more and find products | rndsystems.com/butyrophilins

Learn more and find products | rndsystems.com/gmp

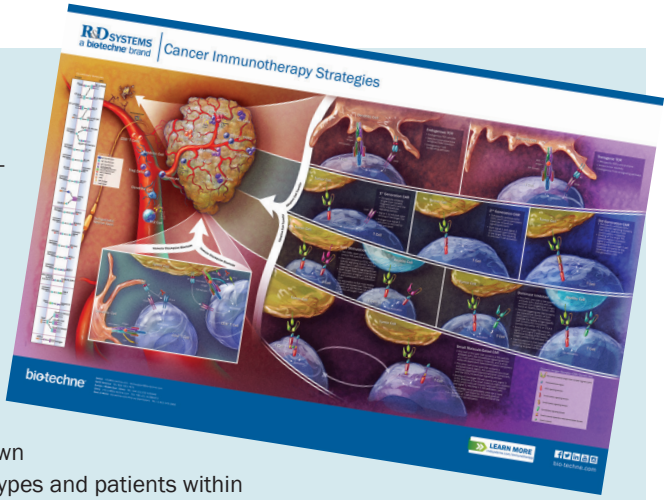
T Cell Co-signaling Molecule Resource

There are a large number of T cell co-inhibitory molecules that could potentially be utilized as targets for combination immune checkpoint blockade strategies. Explore our interactive T Cell Co-signaling Pathway to view T cell co-inhibitory ligand-receptor interactions and view up-to-date product listings.



Explore our interactive pathway | rndsystems.com/pathways_tcellcosignaling

New Wall Poster!



Cancer immunotherapy is showing huge promise as a cancer treatment strategy. This approach aims to utilize the patient's own immune system to target and kill the cancer cells. One barrier to successful cancer immunotherapy is the tumor microenvironment, which recruits immunosuppressive cells that can inhibit endogenous anti-tumor responses. Additionally, endogenous immune responses activate checkpoint pathways that modulate the duration and amplitude of inflammatory responses and minimize damage to healthy tissue. Immune checkpoint blockade is a therapeutic strategy that specifically targets checkpoint pathways with the goal of sustaining anti-cancer inflammatory immune responses. CTLA-4 and PD-1/PD-L1 blockade has shown promise, but has not been a universally successful approach across cancer types and patients within given cancer types. Combination approaches that block multiple immune checkpoint pathways simultaneously may be able to improve therapeutic success. Adoptive T cell therapy (ACT) is another approach to cancer immunotherapy that consists of isolating, expanding, and functionally verifying patient T cells prior to delivering them back into the patient. The use of chimeric antigen receptors (CARs) is a strategy within ACT that directs T cells specifically to cancer cells by targeting tumor-associated antigens. There are several approaches to CAR design and they are outlined in this poster.

Request poster here | rndsystems.com/immunotherapyposter

T Cell Exhaustion

T cell exhaustion (Tex) is a state of dysfunction that results from persistent antigen and inflammation, both of which commonly occur in cancer tissue. The reversal or prevention of exhaustion is a major area of research for cancer immunotherapy. Currently, analyzing multiple phenotypic and functional parameters in combination is required for the identification of Tex populations. A better understanding of T cell exhaustion mechanisms and the identification of unique phenotypic markers for Tex cells will greatly facilitate the advancement of cancer immunotherapies strategies.

Hallmarks of Exhaustion

Hallmarks commonly used to monitor CD4$^+$ and CD8$^+$ T cell exhaustion are listed below. We offer a wide range of products for your workflow, including proteins (GMP-grade available!), ELISAs, flow cytometry antibodies, and assays for proliferation and cytotoxicity.

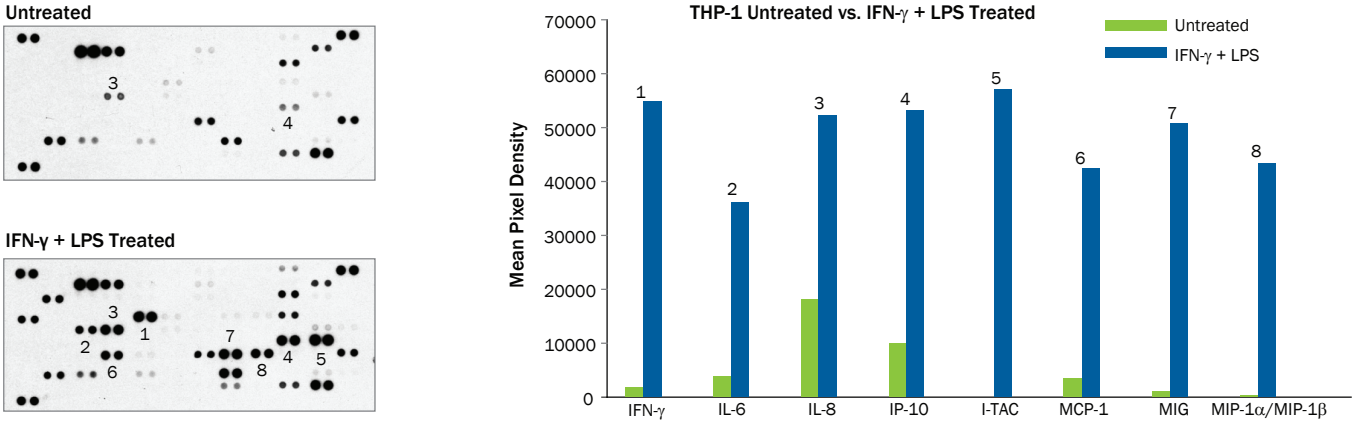
CD8$^+$ T cells

- Co-express multiple inhibitory receptors: PD-1, CTLA-4, LAG-3, TIM-3, 2B4/CD244/SLAMF4, CD160, TIGIT
- Loss of IL-2 production, proliferative capacity, *ex vivo* cytolytic activity
- Impairment in the production of TNF-α, IFN-γ, and cc (beta) chemokines
- Degranulation; expression of high levels of Granzyme B
- Poor responsiveness to IL-7 and IL-15, which drive memory T cell antigen-dependent proliferation long after antigen elimination
- Cell death, likely due to overstimulation

CD4$^+$ T cells

- Co-express multiple inhibitory receptors: PD-1, CTLA-4, LAG-3, TIM-3, 2B4/CD244/SLAMF4, CD160, TIGIT
- Loss of IL-2 production, proliferative capacity, *ex vivo* cytolytic activity
- Impairment in the production of TNF-α, IFN-γ, and cc (beta) chemokines
- Altered expression of GATA-3, Bcl-6, and Helios
  - Few CD4$^+$ Tex cells express EOMES
- Become skewed towards a T Follicular Helper (Tfh) cell phenotype
  - Surface Markers: CD4, CXCR5, ICOS, PD-1
  - Secreted Cytokines: IL-4, IL-6, IL-21
  - Transcription Factors: Bcl-6, IRF4, STAT4
- Show earlier manifestation of dysfunction compared to CD8$^+$ Tex cells

Monitor the Expression of Over 100 Cytokines Simultaneously



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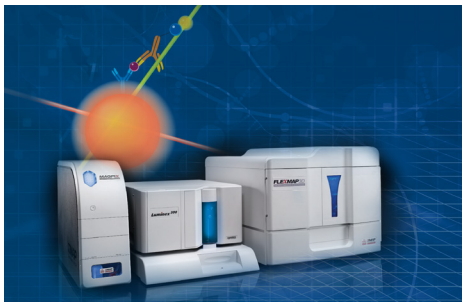

Learn more and find products | rndsystems.com/exhaustion

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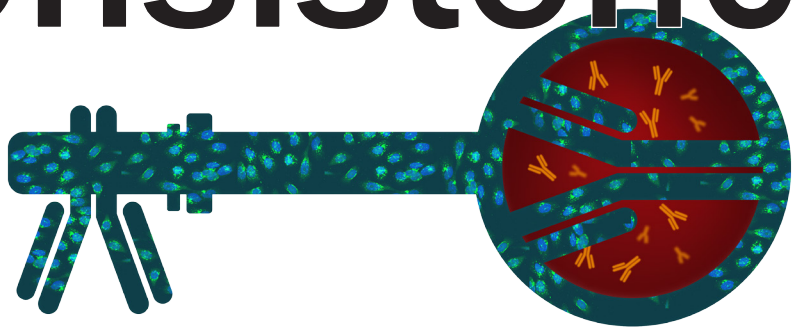
- Bioactivity Testing Services
- Ubiquitin/Proteasome Custom Services

Consistency Is



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