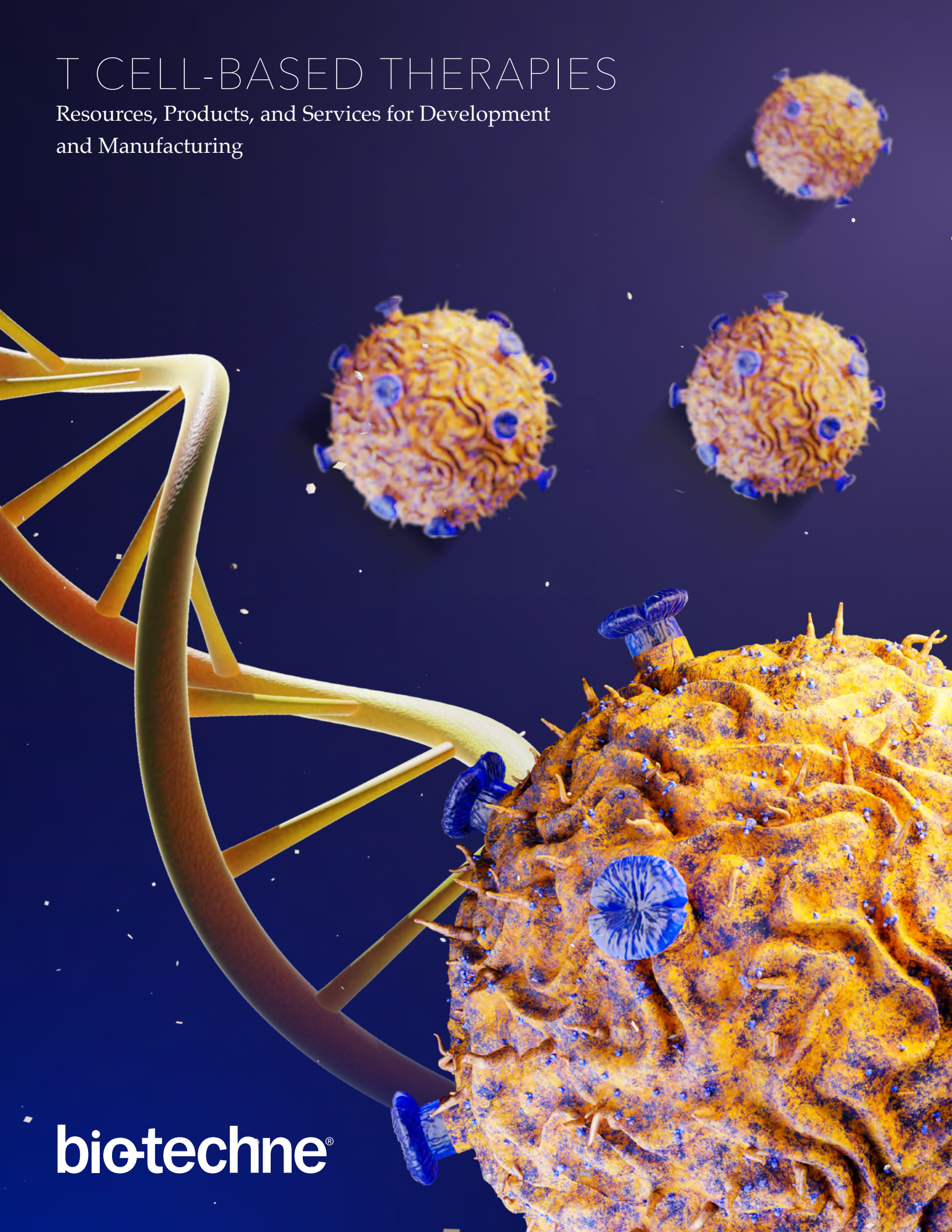
T CELL-BASED THERAPIES

Resources, Products, and Services for Development and Manufacturing

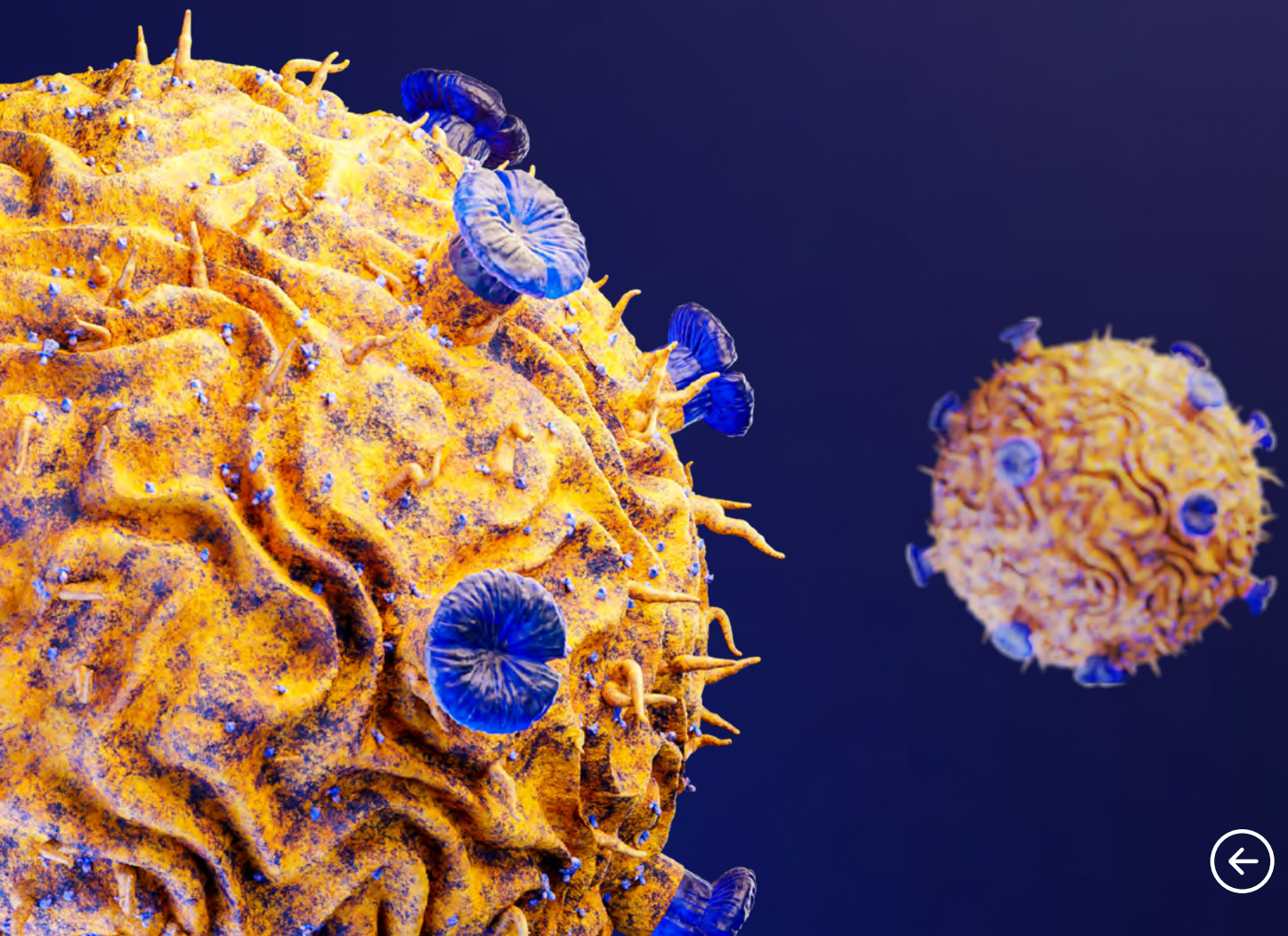


bio-techne®

T cell-based therapies hold dramatic potential for treating intractable diseases by redirecting the power of living immune cells.

The *in vivo* performance of cell therapies will improve with deeper understanding of cellular behavior, while technological advances contribute to process efficiency, scalability, and safety. In this eBook, we outline several of the biological and manufacturing challenges for T cell therapies and highlight how our solutions can help overcome these obstacles at each process stage.

Whether you are at the earliest phase of discovery, looking to move your program into the clinic, or progressing rapidly towards commercialization, there's undoubtedly a Bio-Techne solution for you. From ancillary materials to automated analytical tools, Bio-Techne is committed to delivering innovative solutions that enable cell and gene therapies to reach more patients.



BIOLOGICAL CHALLENGES

Biological challenges arise from the uneven behavior of living cell therapies in a complex *in vivo* environment. These challenges can be addressed through improved understanding of physiological processes, leading to optimized raw material selection, more effective cell engineering programs, and robust cell characterization methods.

1 | T CELL EXHAUSTION

Background and Challenges

For effective T cell therapies, it is important to avoid over-activation of the cells which can lead to T cell exhaustion. It is a delicate balance that can only be attained with thorough investigation of activation conditions and monitoring the activated vs. exhausted state of the cells. This balance may be dependent on the affinity and number of interactions between the T cell therapy and its target cells.

The onset of exhaustion is indicated by the sustained surface expression of co-inhibitory proteins such as PD-1 and CTLA-4. Ligation of these proteins induces intracellular signaling that suppresses cell activation. Individual exhausted T cell subsets may upregulate expression of particular surface and intracellular proteins. $CD4^+$ cells also upregulate these inhibitory proteins with the exception of CD39 expression instead of TIM-3. See the Cell Characterization chapter for tables of activation and exhaustion markers.

Both $CD8^+$ and $CD4^+$ T cells can become exhausted with persistent antigen exposure. This occurs in chronic infection as well as in cancer. Exhausted T cells are less able to participate in immune reactions as shown by reduced proliferation and a progressive loss of effector functions. $CD8^+$ cells secrete less IL-2, TNF-alpha, IFN-gamma, as well as cytolytic proteins such as Granzymes and Perforin. $CD4^+$ cells show reduced cytokine production and reduced ability to activate other immune cells. In addition, exhausted T cells are less responsive to the cytokines that would otherwise enhance their activity, expansion, and survival.

There are at least two distinct populations of exhausted $CD8^+$ T cells. Stem-like cells express low amounts of PD-1 and are susceptible to checkpoint blockade. In contrast, a terminally dysfunctional subset of exhausted $CD8^+$ T cells expresses high levels of PD-1 and is resistant to checkpoint blockade. These cells may still produce Granzyme B and a single cytokine but not respond to activating stimuli.

Regulatory T cells (Treg) can also become exhausted and lose their functionality. It may be possible to exploit this in the tumor microenvironment. See the Tumor Microenvironment chapter.

Overcoming the Obstacles

- Determine and control the optimum cell activation conditions
- Evaluate combinations of activating stimuli and titrate ligand density
- Upregulate or downregulate key receptors with cell engineering
- Rigorously characterize your cells – surface markers and secretory profile

2 | CHECKPOINT BLOCKADE

Background and Challenges

Immune checkpoint proteins play a central role in regulating T cell activation. Tumor cells frequently exploit this system and evade host immune clearance by upregulating ligands that trigger inhibitory receptors. Checkpoint blockade interferes with these interactions by using monoclonal antibodies that target inhibitory T cell receptors. Blockade can restore function and survival of exhausted T cells in the tumor microenvironment.

While checkpoint blockade has demonstrated clinical success, some patients are unresponsive. In addition, some patients initially respond to the blockade but then become resistant by upregulating additional checkpoint proteins. The most commonly targeted inhibitory T cell receptors in this family are CTLA-4 and PD-1. However, several other inhibitory receptor families can also contribute to suppressing anti-tumor immune responses (*e.g.* Butyrophilin, LILRA/B, VSIG, SLAM, and VSTM families).

Inhibitory receptors balance signals transduced through costimulatory proteins such as B7-1/CD80, B7-2/CD86, and CD28. Ligation of these proteins is required for full T cell activation. In addition, costimulatory proteins can interact in cis with inhibitory receptors on the T cell surface, resulting in reduction of their ligand binding ability. For maximum blockade effectiveness, it is beneficial to trigger CD80 and CD86 in addition to binding checkpoint proteins.

Overcoming the Obstacles

- Engineer cells to knock out checkpoint proteins
- Knock out additional checkpoint proteins beyond PD-1 and CTLA-4
- Analyze cells to confirm loss of checkpoint protein expression

CHECKPOINT BLOCKADE RESOURCES

Current and Emerging Immune Checkpoint Targets for Immuno-Oncology Research eBook

Emerging Targets for Cancer Immunotherapy Research Webinar

T Cell Co-Signaling Interactive Pathway: Ligand-Receptor Interactions

Checkpoint Blocking Antibodies

Cancer Immunotherapy Research Brochure

Immuno-Oncology Brochure

Immune Checkpoint Targets for Cancer Immunotherapy Research Poster

Checkpoint Inhibiting Small Molecules

3 | TUMOR MICROENVIRONMENT

Background and Challenges

A tumor with its immediately surrounding area is known as the tumor microenvironment (TME). Tumors develop an immunosuppressive TME that inhibits the host immune system's ability to recognize and destroy tumor cells. The TME is characterized by the recruitment of immunosuppressive cells, activation of immune checkpoint pathways, and exclusion of T cells.

Regulatory T cells (Tregs) are a heterogeneous subset of $CD4^+$ T cells that represent a significant suppressive population in tumors. They inhibit the function of $CD4^+$ and $CD8^+$ effector T cells, natural killer (NK) cells, NKT cells, and antigen-presenting cells. Tregs secrete immunosuppressive cytokines as well as Granzyme A and Granzyme B which induce T cell and dendritic cell apoptosis. See the Cell Characterization chapter for a table of Treg markers.

Cancer associated fibroblasts (CAFs) are activated fibroblasts that suppress anti-tumor immune responses through multiple mechanisms. They promote tumor angiogenesis, fibrosis, ECM remodeling, and tumor progression, and they exclude T cells from the TME. CAFs secrete TGF-beta, IL-6, TDO2, IDO, and VEGF, as well as CXCL12/SDF-1 that polarizes macrophages to an immunosuppressive M2-like phenotype. CAFs are typically identified by the expression of alpha-Smooth Muscle Actin and Fibroblast Activation Protein/FAP as well as FSP1, Vimentin, Desmin, and PDGF R. These proteins are not specific markers for CAFs, but they serve to distinguish them from other cell types in the tumor.

Tumor-associated macrophages (TAMs), like macrophages in other tissues, can be polarized into M1 and M2 phenotypes within the TME. M2-polarized TAMs can suppress T cell and NK cell function by inducing the expression of TIM-3, PD-1, and CTLA-4. They promote angiogenesis, ECM remodeling, and Treg development. They secrete TGF-beta, VEGF, IL-6, and IL-10 and may express checkpoint ligands PD-L1 and PD-L2. In contrast, M1-polarized TAMs can enhance anti-tumor immunity with strong IL-12 production but limited IL-10 production. TAMs also enable tumor growth by expressing SIRP-alpha which binds CD47 on cancer stem cells (CSC) and prevents CSC clearance.

Myeloid-derived suppressor cells (MDSC) comprise a heterogenous population of immature myeloid progenitor cells that fail to differentiate into granulocytes, macrophages, and dendritic cells. MDSC secrete the immunosuppressive cytokines TGF-beta and IL-10, leading to Treg development and the inhibition of NK cell and $CD8^+$ T cell functions. They also can produce Arginase 1/ARG1 and iNOS which inhibit T cell proliferation by disrupting signaling through the TCR and IL-2 receptor. MDSC are $CD11b^+$ $CD14^-$ $CD33^+$ in human and $CD11b^+$ $Gr1^+$ in mouse.

Cancer stem cells (CSCs) are progenitors that reside in the tumor and can differentiate into tumor cells. They exhibit lineage plasticity and broad heterogeneity between patients. These cells maintain stemness through positive feedback from immunosuppressive cells in the TME and potentially in response to ineffective immunotherapy. CSCs recruit TAMs and Tregs to the TME and also promote immunosuppressive M2 macrophage polarization. CSCs secrete IL-4, IL-10, IL-13, and TGF-beta and express CD133, EpCAM, CD90, and CD24.

Adenosine is produced in the TME and exerts immunosuppressive effects on T cells, NK cells, DCs, MDSCs, and macrophages. It promotes tolerogenic macrophage activation through A2b receptors and inhibits inflammatory macrophage activation through A2a receptors.

Overcoming the Obstacles

- Target inhibitory cells in the TME
- Engineer T cells to overexpress stimulatory cytokines, triggered by CAR ligation
- Engineer armored T cells to regulate or sequester suppressive TME cytokines
- Engineer T cells to secrete checkpoint inhibitors (*e.g.* scFV and nanobody)
- Engineer T cells to express T cell engager for tethering to target cells
- Analyze T cell secretory profile and TME cytokines to identify critical signals

TUMOR MICROENVIRONMENT RESOURCES

Mechanisms of Tumor Evasion and Immunosuppression in the Tumor Environment Poster

MDSC-Mediated Mechanisms of Immunosuppression Interactive Pathway

Mechanisms of Regulatory T Cell-Mediated Suppression Interactive Pathway

Mechanisms of Tumor-Associated Macrophage TAM-Mediated Immunosuppression Interactive Pathway

Small Molecules for TME Research

4 | CELL MIGRATION

Background and Challenges

In order for T cell therapies to be effective, the activated cells must reach their target tissue. For targeting solid tumors, peripherally administered cells must first traffic to the tumor, cross physiological barriers to infiltrate the tumor, and persist within the tumor site long enough to eradicate tumor cells. Improvements in trafficking and infiltration can result in more efficacious tumor control. "Cold tumors" can exclude tumor-specific cells from the TME even though they permit infiltration of immunosuppressive cells. See the Tumor Microenvironment chapter.

T cell extravasation from tumor capillaries requires crossing the capillary endothelium as well as the basement membrane. Vascular endothelial cells express E-Selectin, P-Selectin, ICAM-1, ICAM-2, PECAM-1, VCAM-1, VE-Cadherin, and multiple Integrins, each of which plays a role in allowing the T cell to attach to and cross the endothelium. The T cell must express ligands for these proteins for efficient extravasation.

The endothelial cell basement membrane is heterogeneous within tumors and is primarily composed of Entactin, Nidogen, Collagen IV, heparin sulfate proteoglycans (HSPGs), and Laminins. In addition, cancer-associated fibroblasts can lay down dense fibrous networks that T cells must penetrate.

The host's endogenous tumor-specific $CD8^+$ T cells can be actively excluded from the tumor. Their egress from tumor draining lymph nodes (TDLN) is a key step in homing to the tumor. These $CD8^+$ cells may still be functional in contrast to the exhausted cells within the TME. Immune checkpoint blockade can preferentially mobilize functional $CD8^+$ cells to the tumor with CCR5 and CXCR3 as key mediators of the migration.

Conventional dendritic cells (cDCs) within TDLN are often suppressed in cancer. They can be excluded from the tumor by PGE2 and adenosine produced in the TME. Lymph node-resident cDCs are characterized as $CD11c^{high}$, $CD1c^+$, $CD141^+$, $CD14^+$, and $CD1a^-$. Migratory cDCs express CD1a and require the expression of CCR7 for trafficking to the TDLN and participating in anti-tumor responses. cDCs are recruited to the tumor in response to the chemokines CCL4, CCL5, and XCL1 and blocked by tumor-derived PGE2. Within the TME, cDCs are critical for reactivation of central memory T cells and T cell infiltration.

Overcoming the Obstacles

- Confirm that T cells express the necessary extravasation proteins
- Engineer cells to express any lacking chemokine receptors and homing molecules
- Protect cells from host clearance with knockout of CD52 and MHC I proteins
- Engineer cells to express CD47 for blocking NK cell-mediated clearance

CELL MIGRATION RESOURCES

The Vasculature in Inflammation Research Area

Leukocyte Adhesion and Extravasation Research Area

5 | TUMOR HETEROGENEITY

Background and Challenges

T cell therapies often rely on the identification and targeting of antigens expressed by tumor cells but not by normal host cells. Beyond these targets, tumor cells can express tumor-specific antigens (TSAs) which arise from tumor-specific mutations. TSAs are often unique to each patient and therefore require personalized therapies. TSAs are the main drivers of protective $CD8^+$ T cell responses and TIL therapy. In contrast, tumor-associated antigens (TAAs) are encoded in the germline and are not patient-specific. TAAs are weakly responsive to targeted therapy due to central immune tolerance.

Tumors often exhibit non-uniform expression of these antigens which results in variable responsiveness to T cell therapies and antigen escape of some tumor cells. Antigen loss is a common cause of clinical relapse. Antigen heterogeneity can be temporal as well as spatial and can be apparent between the primary tumor site and its metastatic sites.

Antigen presentation requires multiple intracellular components, many of which can be downregulated in tumor cells. Since these are not essential for cell survival, these components can be disrupted while allowing the cell to survive and proliferate. These components include the immunoproteasome, TAP, Tapasin, ERAP1, and beta 2-Microglobulin. They are commonly upregulated by inflammatory signaling based on IFN-gamma stimulation and signal transduction through NF kappa B, IRF1/2, and NLRC5.

In antigen presentation, peptides derived from tumor antigens are presented on the cell surface in a complex with MHC molecules (MHC class I on $CD8^+$ T cells and class II on $CD4^+$ T cells). Tumor cells can polarize dendritic cells to a tolerogenic phenotype and interfere with antigen presentation by inhibiting the expression of several of these components. Loss, downregulation, or mutation of MHC I on tumor cells contributes to immune evasion. Tumor cells can be targeted by NK cells but may respond by expressing non-classical MHC Ib molecules that do not trigger NK cell activation. Since chimeric antigen receptors (CARs) are based on antibody fragments and not TCR, they interact with antigens independently of MHC molecules.

Immune editing refers to changes in tumor cell phenotype in response to immune therapy pressure. The first administration of a T cell therapy may kill all the antigen positive cells but allow antigen negative cells to proliferate. The tumor would then be unresponsive to a second administration of therapy. Similarly, acquired instability develops as therapy administration selects for tumor cells that are more highly prone to genetic mutations. A tumor cell may undergo lineage switch in response to therapy; this cell can give rise to a population that is unresponsive to the therapy.

Overcoming the Obstacles

- Engineer T cells to express bispecific or tandem CARs
- Engineer cells to express multiple CARs
- Identify and engineer CARs with optimum affinity for their antigens
- Administer multiple separate CAR-T cell therapies

6 | T CELL TYPES

Background and Challenges
Adoptive T cell therapies take advantage of the inherent functions of particular types of T cells to fight disease. Autologous therapies rely on cells harvested from the patient, expanded, and re-infused into the same patient, while allogeneic therapies rely on cells harvested from a healthy donor, expanded, and used to treat a different patient. Allogeneic approaches offer the promise of banking cells from "universal donors" which could significantly shorten the time required before the product is ready for patients.

See the Cell Characterization chapter for tables of T cell subset marker antibodies.

Antigen Specific T Cells (CAR-T Cells) are the best characterized and most commonly used cell type for immune cell therapy. Protocols for the isolation, engineering, activation, and characterization of these cells have been defined and optimized in more detail than for other T cell types considered for cell therapies. $CD8^+$ cells are particularly important and kill targets through Perforin or FAS-dependent cytolytic activity as well as by promoting inflammation. Naïve $CD8^+$ cells can expand into short lived effector cells (SLEC) and memory precursor cells (MPECs).

Tumor-Infiltrating Lymphocytes (TILs) are non-circulating cells that typically reside in solid tumors of epithelial origin. They are isolated from excised tumors or biopsies, selected for tumor specificity, and expanded with IL-2 or tumor-derived antigens. The tumor origin of TILs confers strong tumor-homing properties after they are readministered to the patient. A subset of TILs has a T resident memory-like phenotype, typically characterized as $CD69^+$ and $CCR7^-$ (and predominantly $CD103^+$ in the $CD8^+$ population). The polyclonal nature of TIL cultures is advantageous in cases of high tumor heterogeneity or if tumor antigens are not defined. At the same time TIL culture heterogeneity increases the difficulty of consistent and effective gene engineering. The TIL content of tumors is positively correlated with MHC I expression and $CD8^+$ T cell sensitivity.

NKT Cells express the α/β T cell receptor (TCR) but, unlike conventional T cells, they recognize lipids presented by CD1d and are not MHC restricted. This offers the potential to utilize "universal donors" and develop allogeneic therapies. NKT cells can promote anti-tumor immunity by inducing DC maturation and the activation of NK cells and $CD8^+$ cytolytic T cells and also by inhibiting myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). The Vα24-invariant subset of NKT cells can additionally lyse tumor cells through the release of Granzyme B, Perforin, and Fas Ligand. The introduction of chimeric antigen receptors (CARs) provides an additional mechanism for target cell killing, an advantage over conventional CAR-T cells. NKTs express CD16, CD56, and a broad range of cytokines. An L-Selectin/$CD62L^+$ subset of NKTs exhibits a central memory-like phenotype with prolonged persistence and anti-tumor function.

Gamma/Delta T Cells express the gamma and delta chains of the T cell receptor rather than the α/β chains on conventional T cells. γ/δ T cells recognize antigens independently of MHC molecules and therefore hold promise for allogeneic cell therapies. They are active against a broad range of tumor cells, can cross-present antigens to α/β T cells, and can activate NK cell-mediated lysis of inflammatory dendritic cells.

Potential advantages of γ/δ T cells over other cell types

- Tumor cell loss of MHC-I or beta 2-Microglobulin does not prevent targeting by γ/δ T cells
- Different modality compared to CAR-T – doesn't require specific antigen stimulus
- Resistance to checkpoint inhibition – most do not express PD-1
- Killing target cells that lack tumor antigens (needed for CAR or Ab targeting)

The Vδ1 subset is generally resident in mucosal and epithelial tissues. The Vδ2 subset circulates and, of these, Vγ9Vδ2 cells are the dominant subset and show a central and effector memory phenotype. Naïve γ/δ cells are $CD45RA^+$ $CD27^+$; central memory cells are $CD45RA^-$ $CD27^+$; terminally differentiated cells are $CD45RA^+$ $CD27^-$. γ/δ cells can be activated and expanded in multiple ways including TCR crosslinking, IL-15 stimulation, ligation of NKG2D or butyrophilins, and Zoledronate.

Regulatory T Cells (Tregs) provide antigen-specific tolerance and tamp down immune responses. For cell therapy, Tregs offer the promise of restoring immune tolerance and reducing the need for immunosuppressive drugs in autoimmunity, inflammatory disorders, and organ transplant rejection. Tregs can be engineered to express CARs and TCRs directed against HLA molecules or autoimmune antigens. Tregs function by secreting inhibitory cytokines such as IL-10, IL-35, and TGF-beta, suppressing cytokine secretion by Th cells, expressing CTLA-4 to block DC activation, and depriving other T cells of IL-2. Treg cultures are expanded with CD3 and CD28 antibodies, IL-2, and mTOR blockade with rapamycin. Active Tregs exhibit the phenotype $CD4^+$, $CD25^+$, $FoxP3^{high}$, $CD127^{low}$.

IMPORTANT TARGETS FOR T CELL THERAPIES

TARGET	ANTIBODIES	ELISA KITS	SIMPLE PLEX	PROTEINS	LUMINEX	PROTEOME PROFILER
alpha-Smooth Muscle Actin	Yes	-	-	-	-	-
B7-1/CD80	Yes	Yes	-	Yes	-	-
B7-2/CD86	Yes	-	-	Yes	-	-
B7-H3	Yes	Yes	-	Yes	Yes	-
BCMA	Yes	Yes	Yes	Yes	Yes	-
Cadherin 17	Yes	Yes	-	-	-	-
CD7	Yes	-	-	Yes	-	-
CD19	Yes	-	-	Yes	-	-
CD20	Yes	-	-	-	-	-
CD27 Ligand	Yes	Yes	-	Yes	Yes	-
CD30	Yes	Yes	-	Yes	Yes	Yes
CD34	Yes	-	-	Yes	Yes	-
CD38	Yes	Yes	-	Yes	-	-
CD117/c-Kit	Yes	Yes	-	Yes	Yes	-
CD160	Yes	-	-	Yes	-	Yes
EGFR	Yes	Yes	-	Yes	Yes	Yes
EGFR Viii	-	-	-	Yes	-	-
EMMPRIN/CD147	Yes	Yes	-	-	Yes	Yes
EpCAM/TROP-1	Yes	Yes	-	Yes	Yes	-
EphA2	Yes	Yes	-	Yes	Yes	Yes
ErbB2/Her2	Yes	Yes	Yes	Yes	Yes	Yes
ErbB3/Her3	Yes	Yes	-	Yes	Yes	-
Flt-3/Flk-2	Yes	Yes	-	Yes	-	Yes
Glypican 3	Yes	Yes	-	Yes	-	-
IL-3 R alpha/CD123	Yes	-	-	Yes	-	-
IL-13 R alpha 2	Yes	Yes	-	Yes	-	-
Mesothelin	Yes	Yes	Yes	Yes	Yes	Yes
MICA	Yes	Yes	Yes	Yes	Yes	-
MICB	Yes	Yes	-	Yes	Yes	-
MICL/CLEC12A	Yes	-	-	-	-	-
MUC-1	Yes	-	-	-	Yes	Yes
NCAM-1/CD56	Yes	Yes	-	Yes	Yes	-
NKG2D	Yes	-	-	Yes	-	-
PD-1	Yes	Yes	-	Yes	-	Yes
PD-L1/B7-H1	Yes	Yes	Yes	Yes	Yes	-
PSMA/FOLH1	Yes	Yes	-	Yes	-	-
ROBO1	Yes	Yes	-	Yes	-	-
ROR1	Yes	Yes	-	Yes	-	Yes
Siglec-2/CD22	Yes	Yes	-	Yes	-	Yes
Siglec-3/CD33	Yes	-	-	Yes	-	Yes
TIM-3	Yes	Yes	Yes	Yes	-	Yes
TROP-2	Yes	-	-	Yes	-	Yes
WT1	Yes	-	-	-	-	-

T CELL SUBSET RESOURCES

Helper T Cell Markers Interactive Tool

T Cell Subsets Poster

$CD4^+$ T Cell Subsets Brochure

Regulatory T Cells Brochure

Natural Killer Cells Brochure

MANUFACTURING CHALLENGES

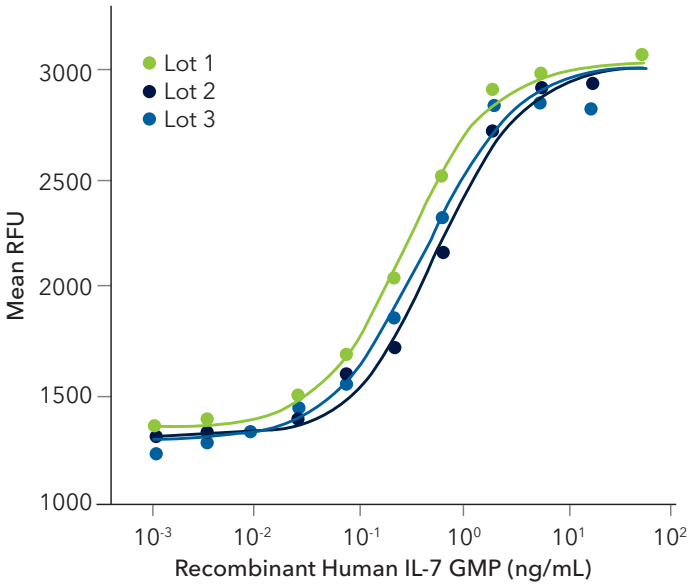
Manufacturing challenges relate to physical production of the cell therapy within the regulatory requirements for therapeutic products. These challenges can be addressed with raw materials and instrumentation designed to increase process efficiency and reduce the risk of batch failures. Advances in the understanding of T cell therapy biology can be most rapidly exploited with streamlined transition from research to manufacturing.

1 | RAW MATERIALS QUALIFICATION

Rigorous qualification of raw materials as well as suppliers will help ensure supply chain reliability for your process. This is a key requirement for standardizing a robust manufacturing process. A supplier should be able to produce materials to your quality specifications and deliver them with your required schedule. All materials should be manufactured and handled in appropriately certified facilities with documented protocols consistent with regulatory requirements.

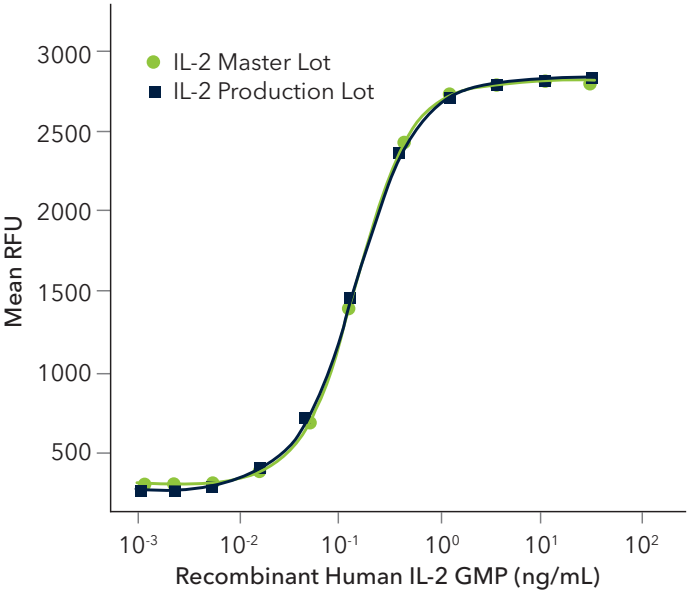
All raw materials used in a T cell therapy manufacturing process (*e.g.* cell culture media, supplements, cytokines and growth factors, antibodies, small molecules, virus vectors) should be qualified for batch-to-batch consistency at the commercially required scale. Materials should be tested with validated quality control assays including bioactivity assays, and these test results should be provided for representative batches of raw materials.

Lot Consistency



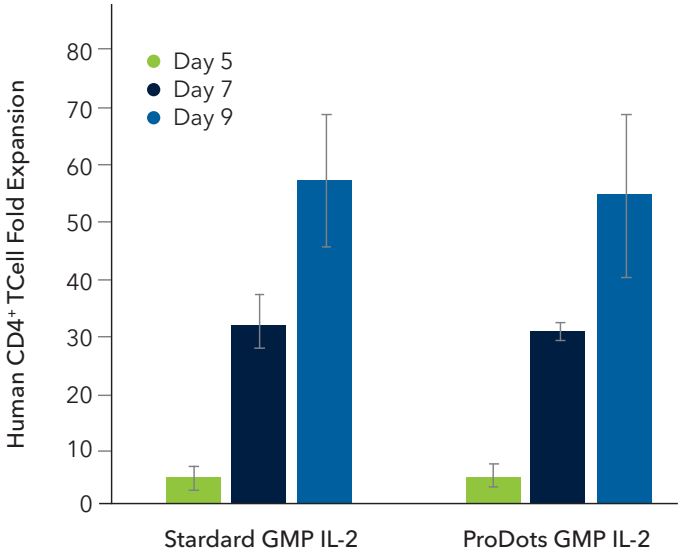
Three independent production lots of Recombinant Human IL-7 GMP Protein were tested for activity in a cell proliferation assay (proliferation of PHA-activated human peripheral blood lymphocytes). Each trace represents a different manufacturing run.

Master Lot Benchmarking



Every production lot should be tested and compared to a master lot to ensure consistency of raw materials over time. This testing controls for variability in the protein as well as in the assay itself, which can both be sources of apparent activity differences when lot bridging. This example considers the bioactivity of Recombinant IL-2 GMP Protein for inducing the proliferation of CTLL-2 cytotoxic T cells.

Formulation Consistency



Manufacturers may have different requirements for raw materials formulation at different steps of their processes. To increase flexibility in process development, we can deliver materials as liquid frozen, lyophilized, or in formulations designed for closed system cell culture. This chart confirms the equivalent bioactivity of ProDots™ Protein and standard formulation of recombinant human IL-2 Both cytokine formulations induce comparable proliferation of T cells from human peripheral blood mononuclear cells when used in combination with ExCellerate™ T Cell Expansion Media and the GMP Cloudz™ T Cell Activation Kit.

RAW MATERIALS QUALIFICATION RESOURCES

Recombinant Protein Quality - Protein Production

Protein Biological Activity

Cytokine Activity Unit Conversion

GMP Proteins with DMF Documentation

Raw Materials Customization Webinar

2 | CUSTOMIZATION AND PROCESS SCALING

Optimizing raw materials to fit your process requirements can provide significant benefits in efficiency, safety, and cost. Customization is important to do early in the process, and it takes on increasing importance as a therapy gets closer to commercialization. It is critical to identify a supplier that can function as a flexible partner to enable you to standardize your process at scale and with consistency. Also critical is a partner that is readily accessible to provide technical and regulatory support throughout your process.

Customization can optimize raw material characteristics and formulations for your process. This includes cytokine construct design, physical and functional characterization, formulation, and stability. You determine what the specifications are, and your suppliers should meet those requirements. Often the exact GMP material you require is not commercially available. Research use only (RUO) materials can potentially be utilized in early trials as long as they are appropriately risk assessed. The additional testing and documentation needed to meet GMP requirements can be handled as a customization request.

Competing factors need to be balanced to effectively scale developmental protocols into robust and efficient processes for the cleanroom. Consider this example of cytokine supplementation from vials containing 1 mg, 25 µg, or 10 µg (optimized for the process). The choice of cytokine packaging options depends on multiple considerations including risk and cost.

Customizing the packaging of a raw material order reduces waste and manufacturing risk and ultimately reduces cost. Wasted material and unnecessary cost are reduced by including the specified amount of material for a given process step in each vial. In addition, customization greatly lessens the manual handling needs which limits the risk of human error in the cleanroom. Bio-Techne offers custom vialing, labeling, packaging to support you in de-risking your process further.

Customizing raw material vialing by activity in international units (IU) instead of mass simplifies media preparation by providing the exact amount necessary in each vial, regardless of material lot. Unless the supplier's raw material is extremely consistent lot-to-lot, vialing by mass generates uncertainty in material bioactivity and requires additional cleanroom steps of calculations and pipetting. Bio-Techne can provide made-to-order (MTO) fills of GMP proteins based on activity, in vials or ProDot Protein™ formulations.

Planning ahead for large-scale manufacturing can lessen future costs and/or future problems by limiting material changes, thus lowering the burden of comparability proof during later stages when it is more expensive. Prioritizing scalability and locking in supply chain reliability early requires a consideration of what would elevate a raw material supplier relationship to an ongoing partnership. Consider master supply and quality agreements to ensure that raw materials arrive when needed and meet exacting specifications.

	1mg VIAL	25 µg "PROCESS" SIZE VIALS		CUSTOM FILL VIALS 10 µg PROCESS SIZE
	ALIQUOT EXACT AMOUNT PROCESS	MEASURE OUT EXACT AMOUNT EVERY TIME	COMPROMISE: USE THE ENTIRE VIAL CONTENTS	EXACT AMOUNT FOR PROCESS
CLEAN ROOM TIME	12	8	4	4
OPTIMAL FOR CELLS?	✓	✓	✗	✓
OPTIMAL FOR PROCESS STANDARDIZATION	✓	✗	✓	✓
RISK	✗✗✗✗✗✗	✓✗	✓✗	✓✗
COST	12	3	4	2

Precision fulfillment of your contracts means receiving raw materials exactly how and when you need them. This results in a more reliable and scalable manufacturing process. Your contracts with suppliers should define materials delivery in addition to specifications for quality, consistency, and bioactivity standardization.

Custom Services at Bio-Techne

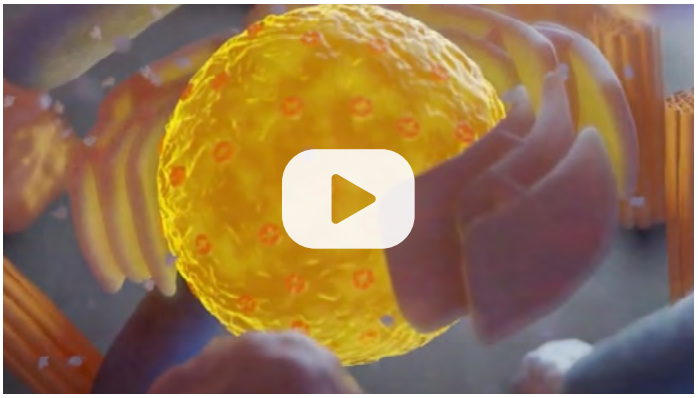
- Custom Services for Cell and Gene Therapy
- Custom Protein Services
- Custom Antibody Services
- Gene Engineering Services
- Custom Cloudz™ Cell Activation Kits
- Custom ELISA Services
- Custom Luminex® Services
- Professional Assay Services
- Custom Compound Library Services
- Custom Chemistry Services

Custom Protein Services

Custom Assay Services

Custom Luminex Services

Custom Antibody Services



Gene Engineering Services

CUSTOM SERVICES RESOURCES

Custom Reagents & Contract Services Brochure

Raw Materials Customizaton Webinar

Streamlining Scale-Up and Scale-Out Webinar

Streamlining Cell Therapy IND Submission Webinar

3 | TRANSITION TO GMP

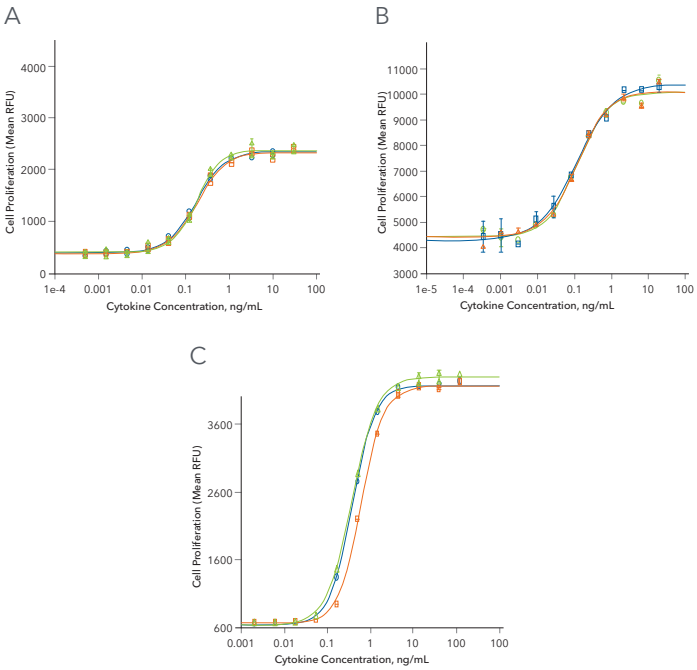
Making the transition from research use only (RUO) to good manufacturing practices (GMP) raw materials does not have to be a big jump. It makes sense to do this early in your process, because more extensive and costly comparability testing is required later during process scaleup. The financial advantages of making the transition early on can outweigh the added cost of GMP materials.

Compared to RUO, GMP-grade raw materials are manufactured under more tightly controlled protocols and are provided with more extensive sourcing and testing documentation. As your T cell therapy approaches commercialization, the need for incorporating GMP-grade materials and services increases.

Partnering with Bio-Techne can make your transition to GMP as smooth and efficient as possible. As we develop GMP reagents, we maintain direct performance comparability between RUO and GMP. This minimizes the chance of unforeseen delays and increases confidence that GMP materials can easily be substituted for RUO. We provide both RUO and GMP grades of specific raw materials from our protein, small molecule, and cell activation lines. In addition, we offer an Ancillary Material grade of small molecules which are manufactured with additional levels of control compared with RUO products and provide an alternative when GMP reagents are not available.

See Analytics Performance chapter for 21 CFR Part 11-compliant instrumentation.

GMP Manufacturing Facility Video



Equivalent bioactivity with RUO, animal-free, and GMP grades of cytokines as measured in cell proliferation assays. (A) RUO, animal-free, and GMP grades of human IL-2 (black, red, green, respectively). (B) RUO, animal-free, and GMP grades of human IL-4 (black, red, and green, respectively). (C) RUO, animal-free, and GMP grades of human IL-6 (black, red, and green, respectively).

GMP RESOURCES

- Streamlining Transition to GMP
- GMP Capabilities at Bio-Techne
- GMP Quality Policy and Regulatory Support
- RUO, GMP, and Ancillary Material Grades of Small Molecules
- GMP Cytokines and Growth Factors for Therapeutic Manufacturing Brochure
- Raw Materials Customizaton Webinar

4 | CELL CULTURE SYSTEMS

The cell therapy manufacturing industry has many technical challenges to overcome, including but not limited to automating final product fill/finish, closed-system cytokine and reagent addition, and cell phenotype consistency during scale-up. One universal challenge for the future success of cell therapy manufacturing is defining a process that is both scalable and commercially viable.

All-in-one closed-system processing platforms offer cell therapy companies an immediate closed and automated solution for cell therapy manufacturing. While realistic during early and small-scale clinical trials, these systems quicky become cost-prohibitive during scale out – burdened with escalating capital equipment costs and an overwhelming requirement for additional space to accommodate high-volume parallel patient processing. In addition, the translation from research to large scale protocols require intensive process development work which puts the consistency and quality of the final product at risk.

Improving unit operation efficiency and reconsidering basic principles of cell culture are critical to finding the right balance between scalability and a commercially sustainable manufacturing process. A few concepts that can make a big impact on this challenge are below:

Eliminate, then automate – All-in-one automated platforms can place unnecessary limitations on manufacturing capacity, at the level of cell yield per system as well as in practicality of scaling out. An alternate path to sustainable manufacturing is to first eliminate scale-limiting complexities at each unit operation, then tie the optimized operational units together through automation.

Uncouple cell culture from cell processing – Unit operations utilizing fit-for-purpose technologies (instruments, bioreactor) allows cell culture to be uncoupled from cell processing. This can greatly increase (up to 10x) the potential patient processing power of a manufacturing process. In this scenario, cell production for dozens of patients occurs in parallel within a single incubator, while optimized cell processing systems are designed to output cells for multiple patients per day, both upstream and downstream of cell expansion. For example, G-Rex bioreactors are designed to provide oxygen and nutrients to cells on-demand, increasing the reliability of cell production and removing the necessity of complex instruments for media exchange.

Use a process that scales-up AND scales-down – A cell production platform that enables the same culture protocol to be used at both small scale and large scale can increase the pace of model development, primary/secondary reagent qualification, and the reproducibility of target cell phenotype during scale-up.

Cell phenotypes can drift during scale up, especially when moving from a flask-based cell expansion system into a gas permeable bag or stirred tank bioreactors. In this case, cell populations and individual phenotypes may vary outside of critical quality attributes (CQAs) defined at small scale. It's key to keep this challenge in mind even before scaling up and to choose a production system that either uses the same protocols for small and large culture batches or requires very slight process changes. Doing so will help reduce process development time, costs, and regulatory headaches that accompany redefining CQAs.

Bio-Techne's partners at ScaleReady adhere to the aforementioned concepts and are focused on helping the industry address these manufacturing challenges. They have assembled a platform that enables a truly scalable and commercially viable manufacturing process for autologous and allogeneic cell therapies.

ScaleReady is a powerful partnership between Bio-Techne, Fresenius Kabi, and Wilson Wolf. Their goal is to provide a platform that helps you seamlessly transition your preclinical research into clinical manufacturing with a focus on scalability and financial sustainability. ScaleReady provides an innovative platform for rapid cell expansion, flexible and fully validated instrumentation, GMP reagents, and GMP engineering services.

Cell Culture and Processing Systems - G-Rex bioreactors are designed to provide oxygen and nutrients to cells on-demand, increasing the reliability of cell production and removing the necessity of complex instruments for media exchange.

The ScaleReady platform is designed around G-Rex Bioreactors. When paired with Lovo and Cue closed-system, automated cell processing systems, the ScaleReady module enables high throughput parallel processing of cell therapies within a small footprint.

Visit | ScaleReady

5 | ANALYTICS PERFORMANCE

Cell therapy manufacturing processes become more reproducible, efficient, and rigorous with improvements in analytic assay performance and instrument automation. Precision, sensitivity, and specificity are central assay parameters for determining if a mid-process or final sample meets critical quality attributes (CQA).

Analytic instrument automation increases assay throughput and reproducibility while reducing manufacturing risk by eliminating manual intervention steps. In addition, automation offers process advantages with ease of operator training, ease of assay transfer between sites, and report generating.

Visit | Analytical Solutions

21 CFR Part 11-compliant software – Using analytical instruments in a GMP environment requires that their operating software is compliant with the FDA Title 21 Code of Federal Regulations (CFR) Part 11. Several of our ProteinSimple™ instrument platforms meet this requirement and offer multiple safeguards for data security including:

- Controlled user login
- Batch control
- Electronic signatures
- Data processing
- Converting and exporting data for third-party software
- Exporting ANDI files to third-party software
- Audit trail and reports
- Archiving raw data with SHA1 hash algorithm encryption

Simple Plex™ Assays on the Ella platform are fully automated ELISAs based on advanced microfluidic circuitry for detecting fragments, oligomers, and host cell proteins with low assay CVs and picogram sensitivity. Single or multianalyte cartridge format options are available and consume 25 µL of sample or less.

- Up to 4 log dynamic range
- Up to 72 samples per run with results in 90 minutes or less
- Samples run in individual channels to eliminate cross-reactivity
- 21 CFR Part 11-compliant

Learn More | Simple Plex

Watch Ella Video

Micro-Flow Imaging™ - MFI quantitates and characterizes contaminating particles in cell culture samples. MFI uses the power of digital microscopy and microfluidics as well as software filters to differentiate between particle types.

- Up to 150 µL/minute at 900,000 particles/mL
- Image-based analysis of subvisible particle morphology based on 10 parameters
- Autosampler for up to 90 samples per run
- High-resolution images with 85% sampling efficiency
- 21 CFR Part 11-compliant

Learn More About MFI

Watch MFI Video

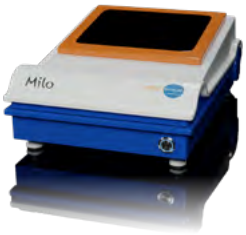
Simple Western™ Systems - The Simple Western family is made up of automated, capillary-based immunoassay platforms that combine the power of CE-SDS or cIEF with the sensitivity of immunodetection, enabling size- and charge-based screening of complex sample types.

- Separate and analyze proteins by either immunoassay or total protein content, from 2 kDa to 440 kDa or from a pI of 3 to 10
- Results from 24 samples in only 3 hours or 96 samples overnight
- Quantitate expression levels, isoform distribution, and fragmentation in a gel- free, blot-free format
- Choose from five instruments of differing throughput and separation mode options
- 21 CFR Part 11-compliant

Compare | Simple Western Systems

Watch Abby Video

Single-Cell Western with Milo™ measures culture heterogeneity with chip-based assays that measuring protein expression and identity in ~1000 individual cells in a single run.



- Multiplex up to 12 proteins simultaneously including multiple isoforms
- Confirm that the expression level of your product is consistent across cells in your culture
- One-minute SDS-PAGE separation on each single-cell lysate
- Chip data acquisition by InnoScan® microarray scanner

Learn More | Single-Cell Westerns

Watch Milo Video

iCE™ Maurice - Maurice is a capillary electrophoresis platform that automates protein profiling by size or charge. Maurice platforms employ pre-assembled cartridges and feature onboard sample mixing. Maurice streamlines cIEF and CE-SDS method development and data analysis for proteins, monoclonals, ADCs, and vaccines.

- Eliminates cross-contamination with separate cIEF and CE-SDS fluid paths
- Whole column imaging by absorbance or native fluorescence
- cIEF charge assay: 100 samples per run at 6 to 10 minutes each
- CE-SDS size assay: 48 samples per run at 25 to 35 minutes each
- 21 CFR Part 11-compliant

Compare | iCE Instruments

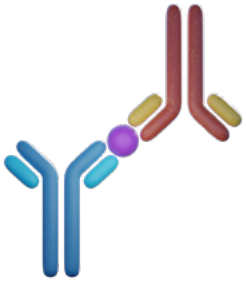
Watch Maurice Video

Exosome Analysis - Analyze gene expression and proteomic profiles to monitor the functionality of your cultured T cells and to screen for biomarkers. Exosomes contain RNA, DNA, and protein components derived from the producing cell and provide an orthogonal readout to complement immunoassay analysis. Our Exosome Diagnostics brand provides robust and highly sensitive assays for targeted expression profiling of gene panels from exosomal RNA derived from subjects' biofluids.

exosomedx a bio-techne brand

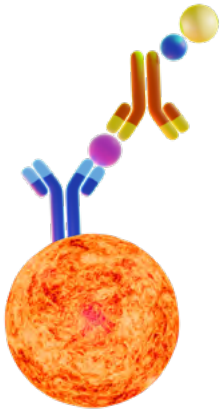
Visit | Exosome Diagnostics

Quantikine™ ELISAs and **Quantikine High Sensitivity ELISAs** are fully validated and optimized immunoassays that undergo a rigorous quality control process to ensure lot-to-lot consistency. They are built with in-house components to help provide unparalleled control over critical elements that affect results and performance over time. Quantikine QuicKit ELISAs maintain the high quality of Quantikine but deliver results in 90 minutes with a simplified workflow. DuoSet ELISAs are a flexible and economical assay development alternative that is adaptable across multiple platforms.



Compare | ELISA Kit Formats

Luminex® assays enable multiplex cytokine profiling for monitoring cytokine release syndrome (CRS). These assays are available as off-the-shelf, curated panels or custom panels built from our selection of over 450 target analytes. These assays maximize multiplexing capacity and flexibility while maintaining target specificity. Luminex profiling up to 50 analytes per sample increases efficiency and improves cost-effectiveness, in either Discovery or High Performance assay formats.



Browse | Luminex Assays

Proteome Profiler™ Antibody Arrays are high throughput, cost-effective tools for early-stage multiplex analyte profiling. They deliver clear and consistent data with superior specificity, low background noise, and no cross-reactivity. Arrays are based on nitrocellulose membranes that are pre-spotted in duplicate with carefully screened, high-quality capture antibodies for multiplexing. They are available for detection of either intracellular or secreted analytes and do not require specialized equipment.

Browse | Proteome Profiler Arrays

6 | CDMO SERVICES

A contract development and manufacturing organization (CDMO) can help get your T cell therapy to the market as quickly and safely as possible. It can be cost effective to contract with a CDMO for their specific expertise, flexibility to meet the demands of scaling, manufacturing capacity, and regulatory support to help get your T cell therapy to the market.

Process Development
Let us scale up your manufacturing process and optimize it for the cleanroom. During process development, we will find out what it takes to turn your program into a reliable process that's robust enough for clinical manufacturing at scale.

- Raw materials selection and supply agreements
- The optimum combination of in-house testing and testing in your hands
- Scheduling time in our GMP manufacturing suites
- Regular process evaluation and troubleshooting to improve inefficient steps

GMP Cell Therapy Manufacturing
When your process is ready for manufacturing, we'll move into our in-house clean room suites for engineering runs and producing your clinical cell or gene therapy. Our GMP facilities are FDA pre-registered, ISO Class 7 certified (Class 10,000) with associated development and quality control labs. You can trust our process as our Quality Team provides regulatory support and oversees facility maintenance, automated batch records, personnel training, and raw materials inspection, testing, and tracing.

Steps in Partnering With Us
Consult with our team to walk through the project step by step with you. During these conversations, we will develop a detailed Statement of Work (SOW) to define

- The project scope and experimental design
- Timelines, milestones, deliverables, project team, and payments
- Custom requirements for deliverables
- GMP manufacturing space availability and scheduling

Following agreement on the SOW, we will deliver a timely quote and be ready to initiate work on the project. At this time we receive materials and all additional relevant information from the customer.

Throughout the project
- Accessibility of project manager or technical lead to facilitate discussions
- Delivery of technical progress reports as defined in the SOW – from project manager or technical lead
- Delivery of cells and other materials for your own testing

Project Completion
- Engineered cells are sent to the customer in pre-defined formulation, # cells/vial, and packaging, and labeling
- Characterization reports, documentation, and other deliverables are sent to the customer as defined in the SOW
- Extra materials are quarantined
- Project is offboarded and archived

Our Strengths for CDMO Partnership
Our in-house team features world class experts to provide you with cutting edge technical understanding. You are getting the best of the best. We connect scientist-to-scientist at all stages of your project – to understand your needs, offer practical recommendations, and facilitate ongoing conversations about the nuts and bolts of the project. We're adaptable and flexible, and we can give you the best materials and the best experience possible.

If you are ready for the next steps, set up a free consultation today.

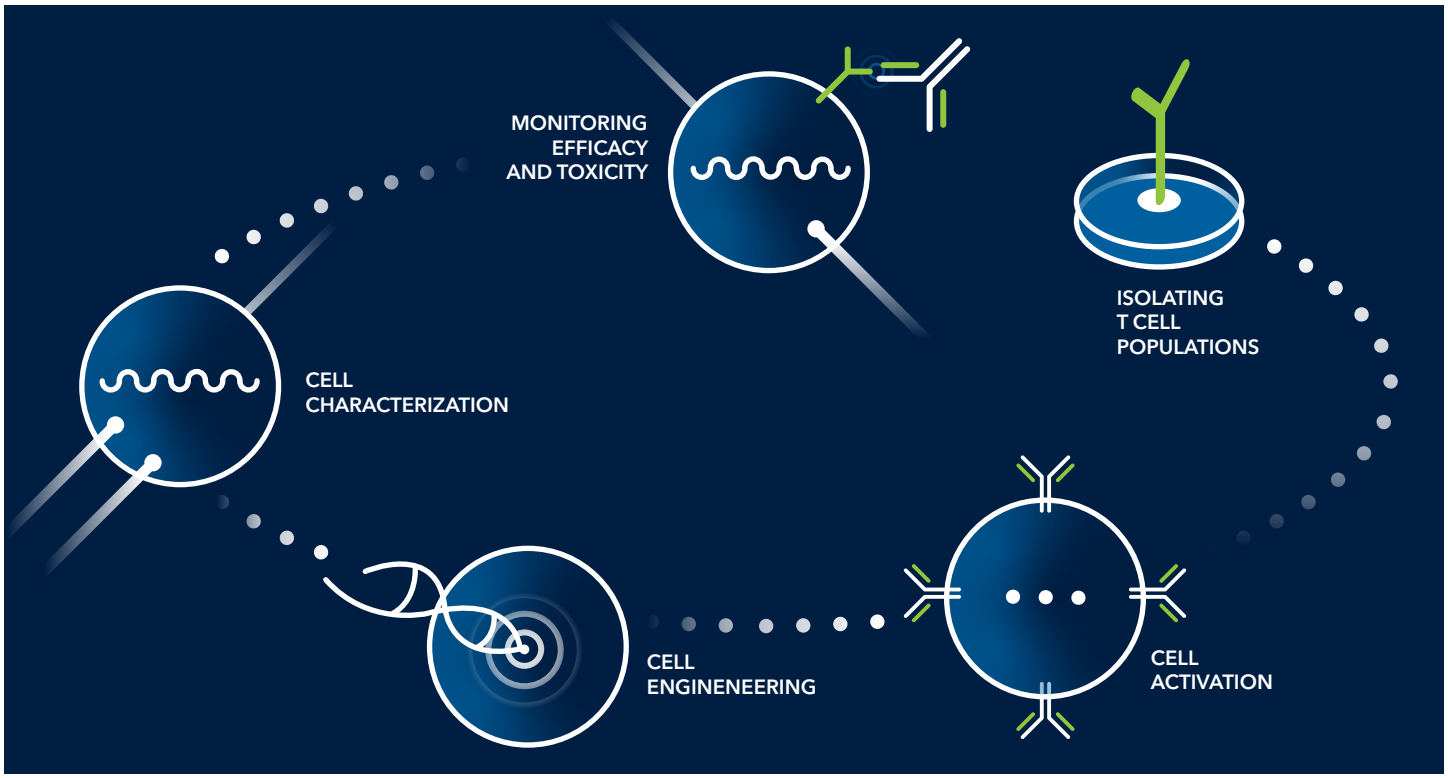
Request a Personal Consultation

Scott Silaika has over 20 years of business development experience in drug development and manufacturing services, spanning discovery through commercial, within the contract development and manufacturing (CDMO) industry. Scott's experience includes numerous examples of identifying, leading, and supporting customer projects on high profile, mission-critical initiatives.

Scott Silaika
Director, Commercial Business Development, Cell and Gene Therapy

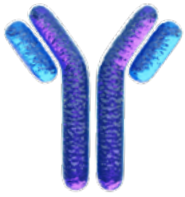
Prior to joining Bio-Techne, Mr. Silaika held commercial development positions at AbbVie, Aesica, Albany Molecular, Avara, and Ricerca Biosciences. He earned his undergraduate degree in Chemical Engineering from Rensselaer Polytechnic Institute and his MBA from the State University at Albany.

PROCESS STEPS



1 | ISOLATING T CELL POPULATIONS

Isolation of particular T cell subsets from a heterogeneous population is the first step to developing new T cell therapies. We offer bead-based kits for the efficient enrichment of T cell subsets with maximum yield and purity. Rigorous analysis of your cell populations for phenotype and viability as well as the lack of contaminating cell types is necessary to confirm that your isolation procedure is effective.

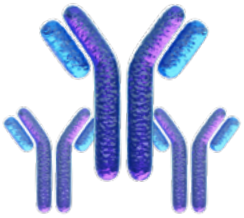


See the chapter on Cell Characterization for more information including tables of surface markers for T cell subtypes.

Download | Immune Cell Therapy Workflow Wall Poster

The Lovo® Automated Cell Processing System facilitates large volume closed system processing of up to billions of cells. Its spinning membrane technology can handle varying cell concentrations up to 22 L sample volumes and deliver final product volumes as low as 50 mL. Lovo automates the labor-intensive task of preparing cells for selection from fresh or frozen leukapheresis with over 90% recovery of target cells.

View | ScaleReady

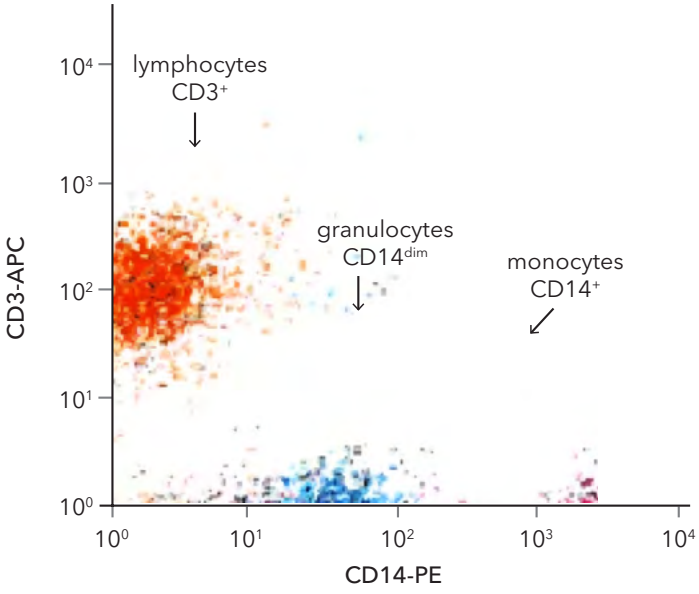


Our Custom Antibodies Services provide highly specific and high quality antibodies for cell selection. Our services include development of monoclonal or polyclonal antibodies, creation of monoclonal antibody panels, production of recombinant antibodies, antibody conjugation, characterization, manufacturing and GMP conversion, anti-idiotype antibody development, and custom engineering services.

Visit | Custom Antibody Services

Our Erythrocyte Lysing Kits enable red blood cell (RBC) clearance from whole blood, which is an important initial step in the isolation and analysis of enriched leukocyte preparations. Our kits lyse erythrocytes under conditions that do not disrupt lymphocytes or myeloid cells which is critical for T cell therapies utilizing leukocytes from whole blood.

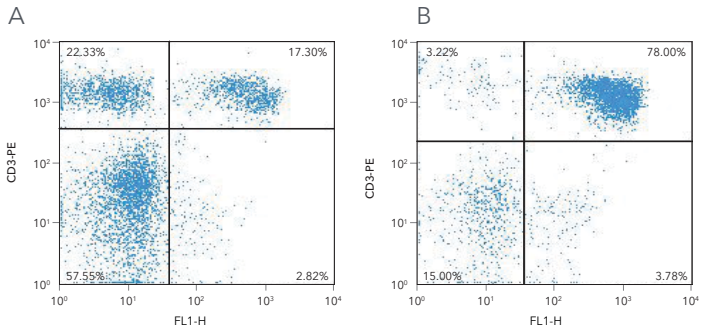
Browse | Erythrocyte Lysing Kits



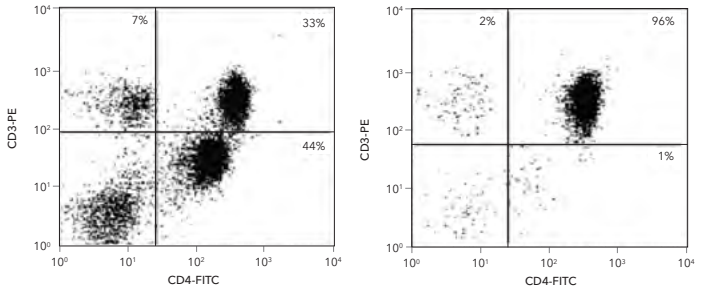
Flow cytometry scatter plot of whole human blood stained with Human CD14 PE-conjugated antibody and Human CD3 epsilon APC-conjugated antibody followed by treatment with the Human Erythrocyte Lysing Kit.

MagCellect™ Cell Selection Kits deliver pure populations of cells by positive or negative selection that can be further characterized and used to research and develop new T cell therapies. They are based on beads that do not induce cell damage and have no magnetic memory in ferrofluid. MagCellect kits separate cells to very high purity in minutes and do not require the use of specialized columns.

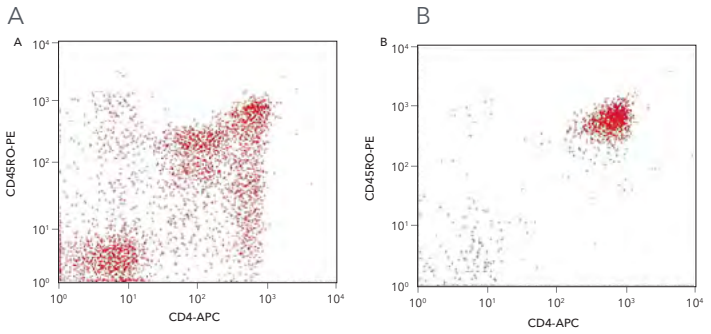
Browse | MagCellect Cell Selection Kits



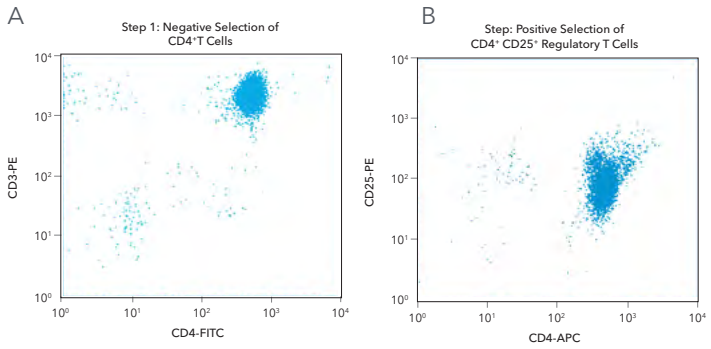
Ficolled human PBMCs before (A) and after (B) isolation of CD8+ T cells using the MagCellect Human CD8+ T Cell Isolation Kit. Dot plots reflect double-staining of all viable cells with Human CD8 alpha Fluorescein-conjugated Antibody and Human CD3 epsilon PE-conjugated Antibody.



Enrichment of CD4+ T cells from human PBMCs with the MagCellect Human CD4+ T Cell Isolation Kit. Cells were stained with a PE-Conjugated Anti-Human CD3 Monoclonal Antibody and a Fluorescein-Conjugated Anti-Human CD4 Monoclonal Antibody before (left) and after (right) T cell isolation.



Human PBMCs (A) before and (B) after isolation of memory CD4⁺ T cells using the MagCellect™ Human Memory CD4⁺ T Cell Isolation Kit. Dot plots reflect double staining of all viable cells with Human CD4 APC-conjugated Antibody and anti-CD45RO-PE antibody.



Enrichment of CD4⁺CD25⁺ T cells from PBMCs. In Step 1, CD4⁺ T cells were isolated from PBMCs by negative selection. In Step 2, CD4⁺CD25⁺ T cells were isolated by positive selection from the CD4⁺ cells recovered in Step 1 by using Anti-Human CD25 Biotinylated Antibody. Cells were stained with Human CD3 epsilon PE-conjugated Antibody, Human CD4 FITC-conjugated Antibody, and Human CD25 PE-conjugated Antibody and analyzed by flow cytometry.

CELL ISOLATION RESOURCES

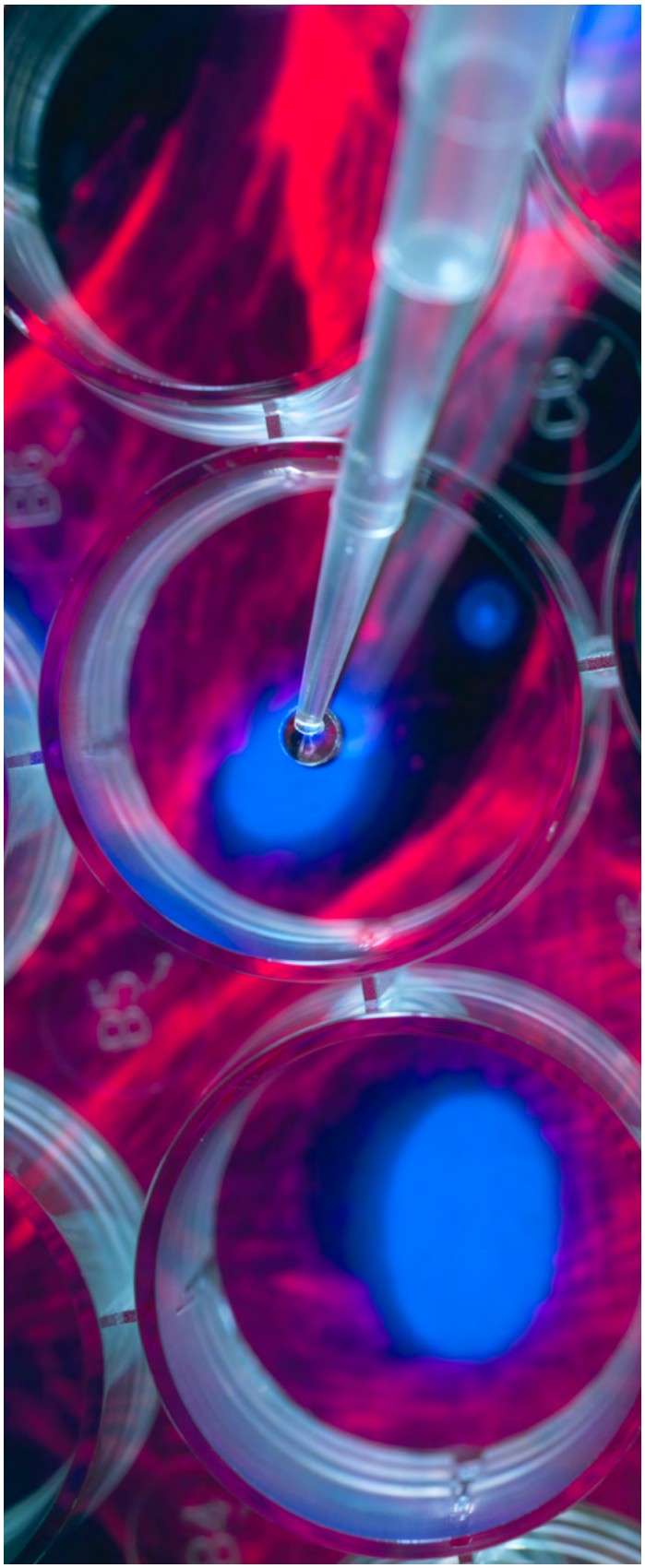
Immune Cell Isolation & Culture Brochure

MagCellect Assay Principle

MagCellect Kit Troubleshooting Guide

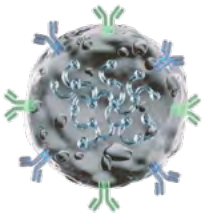
Immunology Protocols

Immune Cell Therapy Workflow Wall Poster



2 | CELL ACTIVATION AND EXPANSION

Expanding T cell cultures from the relatively small size of harvested samples to clinically effective scale requires optimized growth media and equipment. Ancillary materials including media and supplements should be GMP-grade and standardized for activity and reproducibility. The accurate addition of precise levels of growth factors should be confirmed with specific immunoassays. It is critical to achieve robust cell proliferation and activation and also to guard against overactivation and T cell exhaustion.



See the chapters on T Cell Exhaustion and Cell Characterization for more information. See the Cell Culture Systems chapter for a discussion of minimizing cleanroom hands-on time and reducing sources of error.

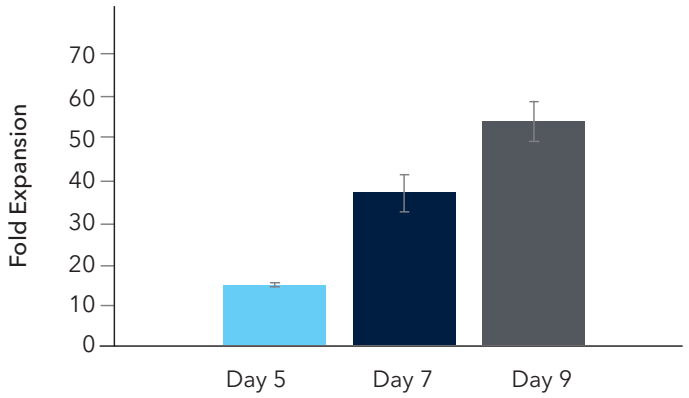
HUMAN CYTOKINES AND IMMUNOASSAYS FOR T CELL EXPANSION MEDIA

CYTOKINE	RUO PROTEIN	GMP PROTEIN	PRODOTS	ELISA	SIMPLE PLEX
IFN-gamma	Human	Yes	Yes	Yes	Yes
IL-1 beta	Human	Yes	Yes	Yes	Yes
IL-2	Human	Yes	Yes	Yes	Yes
IL-4	Human	Yes	Yes	Yes	Yes
IL-6	Human	Yes	Yes	Yes	Yes
IL-7	Human	Yes	Yes	Yes	Yes
IL-12	Human	-	-	Yes	Yes
IL-15	Human	Yes	Yes	Yes	Yes
IL-18	Human	-	-	Yes	Yes
IL-21	Human	Yes	-	Yes	-
IL-23	Human	-	-	Yes	-
IL-27	Human	-	-	Yes	-
TGF-beta 1	Human	Yes	Yes	Yes	Yes
TNF-alpha	Human	Yes	-	Yes	Yes

These assays are matched to the recombinant protein so you get accurate and precise quantitation of the amount of cytokine added to your cell culture media.

Cloudz™ Cell Activation Kits are based on microspheres composed of an alginate-based hydrogel. They are available in multiple sizes and can be functionalized with a variety of ligands including antibodies, proteins, and small molecules. Derivatized Cloudz reagents present solid phase bound ligands to cells to induce receptor crosslinking and intracellular signaling. The hydrogel quickly dissolves when exposed to the release buffer, allowing for gentle and efficient removal of the microspheres. Cloudz kits are available in GMP grade, investigational Screening Cloudz, and custom designed kits. Cloudz for T cells and Treg cells are available through our ScaleReady partnership.

Browse | Cloudz Cell Activation Kits

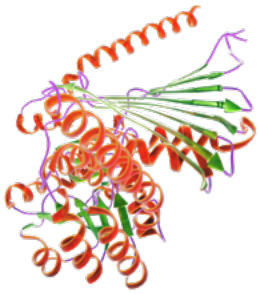


Primary human CD3+ cells were activated with GMP Cloudz™ Human T Cell Activation Kit and cultured for 9 days in ExCellerate™ T Cell Expansion Media and 20 ng/mL GMP IL-2. Cell counts were performed to determine fold expansion compared to the Day 0 seeding density (0.25 x 10^6 cells/mL).

CLOUDZ RESOURCES

Using Micro-Flow Imaging to Assess Activation Bead Removal Application Note

Our GMP Recombinant Proteins are manufactured under guidelines that allow for their use as ancillary materials in cell therapy manufacturing processes. Our GMP-grade proteins frequently originate from the same clone, sequence, and expression system as our traditional research-grade materials. This helps make the transition from basic research into process development and clinical manufacturing as efficient and seamless as possible. GMP Proteins are available through Bio-Techne and our ScaleReady partnership.



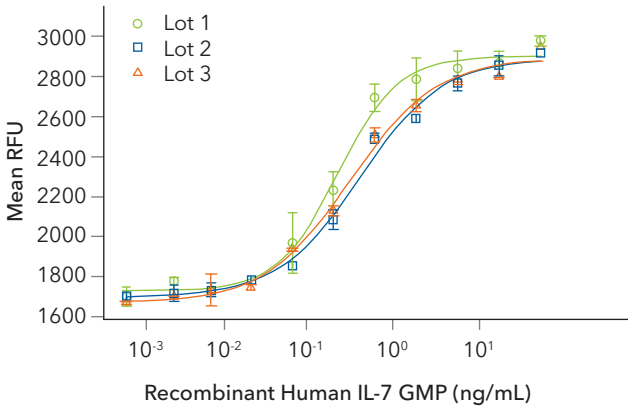
Browse | GMP-grade Recombinant Proteins

GMP PROTEIN RESOURCES

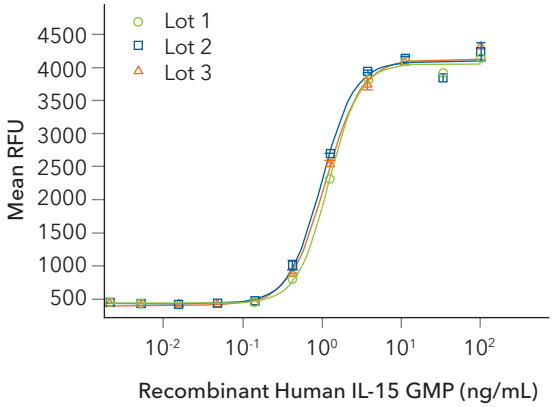
Learn About Our New GMP Manufacturing Facility

GMP Cytokines and Growth Factors for Therapeutic Manufacturing Brochure

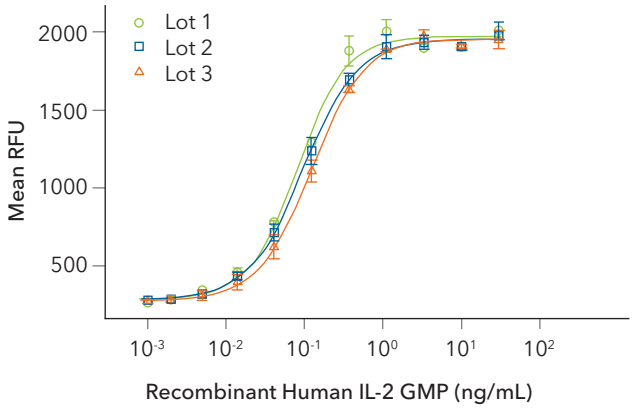
Three independent lots of each of these proteins were tested for bioactivity and plotted on the same graph to show lot-to-lot consistency.



Recombinant Human IL-7 GMP Protein stimulates proliferation of PHA-activated human peripheral blood lymphocytes.



Recombinant Human IL-15 GMP Protein stimulates cell proliferation in the MO7e human megakaryocytic leukemic cell line.



Recombinant Human IL-2 GMP Protein stimulates cell proliferation of the CTLL 2 mouse cytotoxic T cell line.

Quantification of Cytokine Concentrations in Media - After you prepare your cell culture medium, it is critical to confirm that cytokines and growth factors are present at the desired concentrations. We offer a variety of immunoassay platforms that deliver quantitative and reproducible results.

See the Analytics Performance chapter for more information.

Quantikine ELISAs and Quantikine High Sensitivity ELISAs are fully validated and optimized immunoassays that undergo a rigorous quality control process to ensure lot-to-lot consistency. They are built with in-house components to provide unparalleled control over critical elements that affect results and performance over time. Quantikine QuicKit ELISAs maintain the high quality of Quantikine but deliver results in 90 minutes with a simplified workflow. DuoSet ELISAs are a flexible and economical assay development alternative that is adaptable across multiple platforms.

Browse | Quantikine ELISAs

ELISA RESOURCES

The ELISA Guide

Custom ELISA Services

Quantikine ELISA Validation

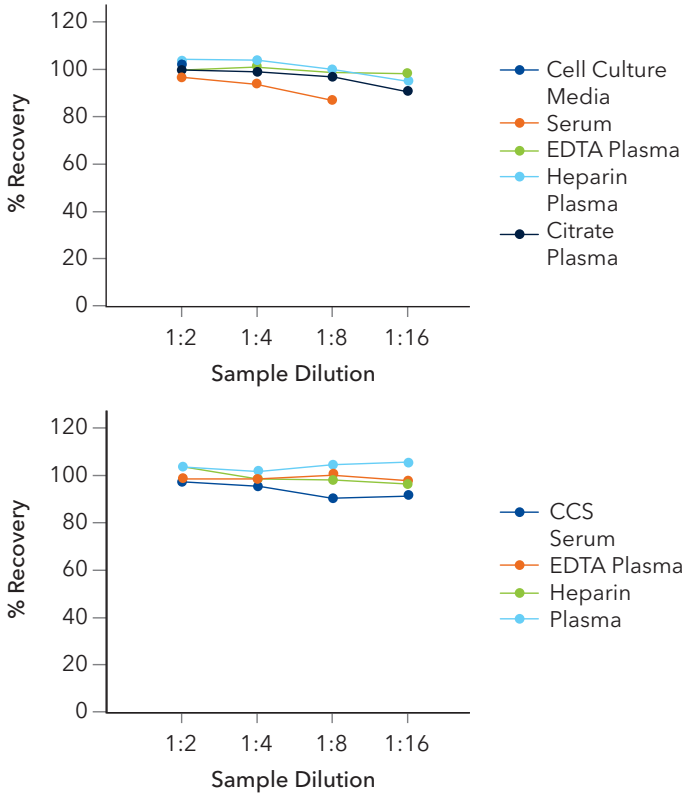
Avoid False Positive Data Application Note

DuoSet ELISA Development Systems

Quantikine QuicKit ELISAs

Simple Plex™ Assays are fully automated immunoassays that offer picogram sensitivity, up to 4 logs of dynamic range, and results in 90 minutes or less. Simple Plex assays minimize hands-on time with built in automation that enables consistent data across multiple users and site locations. Microfluidics cartridges run samples in individual channels to eliminate cross-reactivity and are available in single-plex and multi-analyte configurations.

Browse | Simple Plex Automated Immunoassays



The Quantikine Human IL-2 ELISA (top) and Simple Plex Human IL-2 Immunoassay (bottom) maintain excellent linearity of dilution in cell culture supernates (CCS), serum, and plasma.

ExCellerate™ Human T Cell Expansion Media provides a stable and optimized environment for T cell expansion. It does not contain any non-human animal-derived products and is available without phenol red. ExCellerate media is available bottled or as custom bagged media for closed system processing. ExCellerate media is available through our ScaleReady partnership.

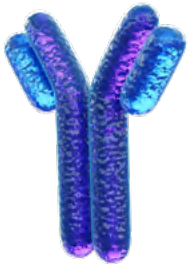
Visit | ExCellerate Media

CELL CULTURE RESOURCES

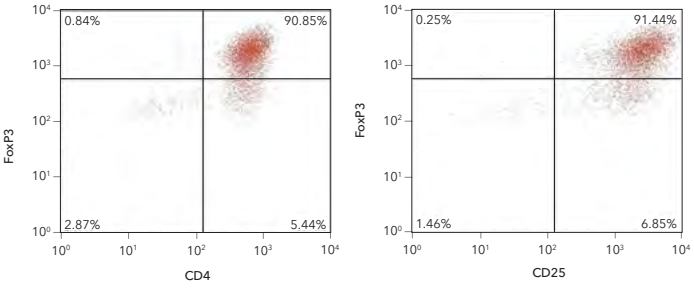
Cell Culture Reagents

Immune Cell Isolation & Culture Brochure

Antibodies for T Cell Activation and Expansion - Trigger activating receptors, block inhibitory receptors, or neutralize inhibitory soluble factors to achieve optimum cell expansion. We offer rigorously tested antibodies validated for agonist or blocking activity. Each antibody is selected based on its performance in carefully chosen bioassays developed and run by in-house scientists.

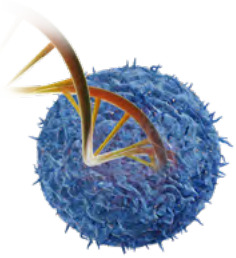


Browse | Antibodies Validated in Functional Assays



Differentiation of human CD4+ T cells into Treg cells confirmed by FoxP3 and CD25 expression. Human peripheral blood naïve CD4+ T cells were incubated with reagents included in the CellXVivo™ Human Treg Cell Differentiation Kit for 5 days. Cells were fixed, permeabilized, and stained using the FlowX™ Human Regulatory T Cell Multi-Color Flow Kit.

3 | CELL ENGINEERING



Cell engineering enables you to fine tune the effectiveness of your T cell therapy by introducing and deleting molecules that alter cell phenotype and function. There are multiple techniques for cell engineering including CRISPR/Cas9, viral transduction, and non-viral methods (*e.g.* TcBuster).

Engineering your T cells can increase their performance in the tumor microenvironment.

- Targeting identified tumor cell markers with chimeric antigen receptors (CARs)
- Overcoming tumor heterogeneity by targeting multiple antigens with bispecific CARs
- Overcoming immunosuppression by deleting or inactivating checkpoint inhibition molecules
- Boosting anti-tumor immunity by overexpression of cytokines
- Minimizing off-target toxicity by disrupting the endogenous T cell receptor

Visit | Gene Engineering Services

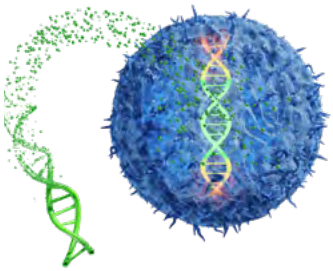
See the Biological Challenges section and Cell Characterization chapter for more information.

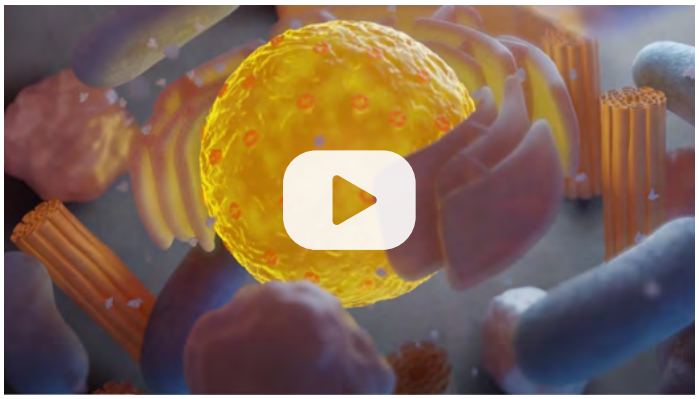
CELL ENGINEERING RESOURCES

GMP Cell Engineering and Cell Processing Services

Genome Engineering Services Brochure

The TcBuster Gene Delivery System is a non-viral platform that enables the stable development of CAR-T cells. The TcBuster transposon-based system supports rapid cell engineering by enabling multigene transfer and CRISPR-mediated knockouts in one operation. TcBuster is available through our ScaleReady partnership.





The advantages of TcBuster over viral transduction include

- Reducing the time required and the cost of introducing a gene of interest (GOI)
- Increasing the practical GOI cargo capacity compared to virus-based methods
- Avoiding inconsistent reagent availability

Learn About | TcBuster Gene Delivery System

CRISPR-Enhancing Reagents can enhance the efficiency of the CRISPR-mediated gene editing process. The CRISPR process introduces double-stranded breaks (DSBs) in DNA which are repaired by endogenous non-homologous end-joining (NHEJ; typically 20-60% efficiency) or homology-directed repair (HDR; typically 0.5-20% efficiency).

PRODUCT NAME	DESCRIPTION
BRD 0539	Cell permeable and reversible Cas9 inhibitor; allows dose and temporal control of Sp-Cas9-based systems
Brefeldin A	Enhances CRISPR-mediated homology-directed repair (HDR) efficiency in human induced pluripotent stem cells (iPSCs)
(Z)-4-Hydroxytamoxifen	Activates intein-linked inactive Cas9, reducing off-target CRISPR-mediated gene editing; metabolite of tamoxifen
KU 0060648	Enhances HDR efficiency and attenuates non-homologous end-joining (NHEJ) frequency
Nocodazole	Enhances HDR efficiency; also increases Cas9-mediated gene editing frequencies
NU 7441	Enhances HDR efficiency and attenuates NHEJ frequency
SCR7 pyrazine	Enhances HDR efficiency

Browse | CRISPR-Enhancing Reagents

Viral Transduction Enhancers can reduce the amount of adeno-associated virus (AAV) or lentivirus required for transduction of cell types that are difficult to infect. Our small molecules target a variety of cellular processes to facilitate viral gene expression, including gene transcription, cell entry, or DNA replication.

Browse | Viral Transduction Enhancers

NAME	DESCRIPTION
Akt-1/2	Enhances CAR and TCR retroviral transduction of human T cells; also potent and selective dual Akt1 and 2 inhibitor
BX 795	Enhances lentiviral transduction of NK cells; also PDPK1 (PDK1) inhibitor
Cyclosporin A	Enhances lentiviral transduction; also calcineurin inhibitor
Cyclosporin H	Enhances lentiviral transduction
Dexamethasone	Enhances retroviral transduction; also anti-inflammatory glucocorticoid
16,16-Dimethyl Prostaglandin E_2	Enhances lentiviral transduction; synthetic prostaglandin E2 derivative
Eeyarestatin I	Enhances AAV transduction
Etoposide	Enhances adenoviral transduction; topoisomerase II inhibitor
MG 132	Enhances AAV transduction efficiency of human cell lines
Prostaglandin E_2	Enhances lentiviral transduction; endogenous prostanoid
Rapamycin	Enhances lentiviral transduction; mTOR inhibitor and immunosuppressant
Rosuvastatin calcium	Enhances lentiviral transduction of NK cells HMG-CoA reductase inhibitor
SAHA	Enhances plasmid transduction; class I and II HDAC inhibitor
Staurosporine	Enhances lentiviral transduction; non-selective protein kinase inhibitor; non-selective protein kinase inhibitor
Teniposide	Enhances adenoviral transduction; DNA topoisomerase II inhibitor

Our Vector Characterization and Quantitation analytical instruments provide the automation and scalability necessary for viral vector development and manufacturing. These platforms can be seamlessly transferred between labs and project phases, enabling consistent vector characterization across locations and from development through manufacturing.

See the Analytics Performance chapter for more information.

VECTOR CHARACTERIZATION RESOURCES

Instrumentation for Vector Characterization

Simple Plex Assays for Viral Titer Quantification

RePlex - One Western Two Immuoassays

Concentrating on AAV Impurities with Ultrasensitive Total Protein Detection on Simple Western Application Note

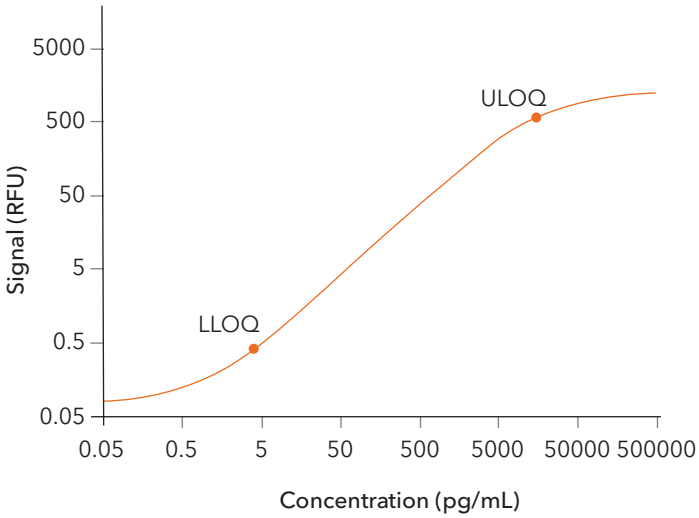
Characterization of AAV Vector Proteins Using Maurice CD-SDS Application Note

icIEF Analysis of AAV Proteins for Gene Therapy Application Note

AAV Characterization and Biodistribution Webinar

Simple Plex™ Assays accurately quantify the titer of lentivirus and adeno-associated virus capsids. Simple Plex assays are fully automated immunoassays that offer picogram sensitivity, up to 4 logs of dynamic range, and results in 90 minutes or less. Simple Plex assays minimize hands-on time with built in automation that enables consistent data across multiple users and site locations. Microfluidics cartridges run samples in individual channels to eliminate cross-reactivity and are available in single-plex and multi-analyte configurations.

Browse | Simple Plex Automated ELISAs



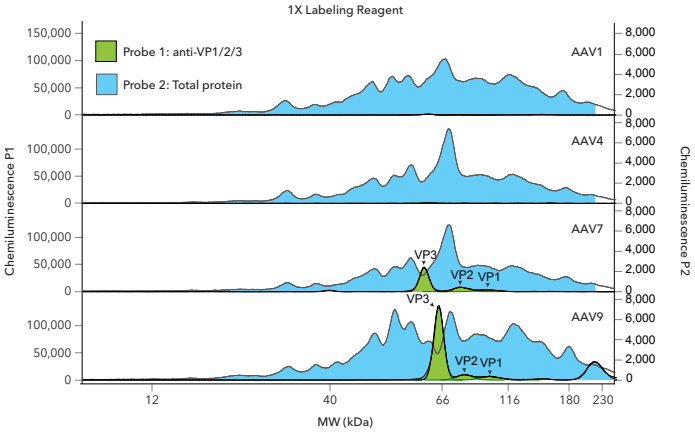
This factory-generated 5PL calibration curve averages 5 replicates of each calibrator from multiple runs. Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) for HIV-1 Gag p24. The limit of detection (LOD) of HIV-1 Gag p24 is 0.67 pg/mL.

iCE™ Maurice is a capillary electrophoresis platform that distinguishes between empty, full, and partially full virus capsids with size and charge based assays run in pre-assembled cartridges. Maurice streamlines cIEF and CE-SDS method development and data analysis with onboard automation and sample mixing.

Compare | iCE Instruments

Simple Western™ Systems enable the characterization of viral vectors with fully automated Western blot analysis. Simple Western capillary-based immunoassays combine the power of CE-SDS or cIEF with the sensitivity of immunodetection, enabling size- and charge-based screening of complex sample types.

Compare | Simple Western Systems



AAV immunoassay analysis with RePlex™ on Simple Western. Overlaid electropherograms of VP1/2/3 protein detection in crude in-process samples with the anti-VP1/2/3 antibody in the first probing cycle (green peaks) and total protein detection in the second probing cycle (blue peaks).

DILUTION	PARAMETER	CCS (N=4)	SERUM (N=4)	EDTA PLASMA (N=4)	HEPARIN PLASMA (N=4)
1:2	Avg % of expected	96	96	97	95
	Range (%)	92-102	91-98	92-105	89-100
1:4	Avg % of expected	97	94	95	93
	Range (%)	90-109	90-99	92-101	90-94
1:8	Avg % of expected	94	95	97	92
	Range (%)	90-99	88-98	89-108	88-96
1:16	Avg % of expected	96	95	98	91
	Range (%)	90-110	87-104	88-111	83-100

Lentiviral vector quantification with Simple Plex. The Simple Plex HIV Gag p24 assay is an automated, reproducible assay that minimizes user error and maintains excellent linearity of dilution in cell culture supernates (CCS), serum, and plasma.

4 | CELL CHARACTERIZATION

Cell therapy quality control has to be robust and consistent, because the cells are intended for human administration. The performance of QC methods must not jeopardize patient safety in any way. It is essential to confirm that cell products meet all critical quality attributes (CQA) at each step of the process. Cell therapies are classified as Advanced Therapy Medical Products (ATMPs) which are significantly more complex than purified pharmaceutical or biologic molecules. As living cellular treatments, they require more extensive characterization to assure efficacy and patient safety.

Rigorous characterization methods reduce the risk of process failures that can arise if substandard cells are passed to the next stage of the process. These failures are extremely expensive and also prevent therapies from quickly reaching the patients who need them.

Critical quality attributes (CQAs) are important to define early in process development and build into the manufacturing process. CQAs are the quality control parameters that are required for a product to pass to the next stage. They should reflect the cell therapy's clinical indications to provide the most accurate indication possible of the product's performance and safety. No one test can define the total quality attributes of a cell product. Orthogonal CQA testing methods analyze unrelated parameters of the product and may include physical characterization of the cells (*e.g.* surface phenotype, activation status, and viability), secretory profile, functional assays (e.g. cytolytic activity), and purity (*e.g.* undesired cell types and particulates).

Visit | Analytical Solutions for Cell and Gene Therapy

CELL CHARACTERIZATION RESOURCES

Challenges of Analyzing ATMPs Webinar

Analytical Instrumentation Solutions for CGT eBook

Investigating Immuno-Oncology: Advances in Protein Analysis Tools

Immunology Protocols

Immunoassay Workflow Solutions Guide

Flow Cytometry Antibodies for T Cell Markers

NAÏVE T CELLS	EFFECTOR MEMORY T CELLS	CENTRAL MEMORY T CELLS
CCR7$^+$	CCR7$^-$	CCR7$^+$
CD45RA$^+$	CD45RA$^-$	CD45RA$^-$
CD45RO$^-$	CD45RO$^+$	CD45RO$^+$
L-Selectin/CD62L$^+$	L-Selectin/CD62L$^-$	L-Selectin/CD62L$^+$

REGULATORY T CELLS	CD8$^+$ T CELLS	GAMMA/DELTA T CELLS
5′ Nucleotidase/ CD73$^+$	CD3$^+$	CD3$^+$
CD3$^+$	CD8$^+$	CD4$^+$
CD4$^+$	CXCR3$^+$	CD8α$^+$
CD5$^+$	Fas Ligand$^+$	CD27 Ligand/TNFSF7$^+$
CD14$^-$	Integrin αLβ2/LFA-1$^+$	CD38
CD19$^-$	Integrin αL/CD11a$^+$	-
CD25/IL-2 Rα$^+$	Integrin β2/CD18$^+$	CD161$^+$
CD39/ENTPD1$^+$	LAMP-1/CD107a$^+$	CD277/BTN3A1
CD103/Integrin αE$^+$		CXCR4$^+$
CTLA-4$^+$		DNAM-1/CD226
GITR$^+$		Fas Ligand
IL-7 Rα/CD127^{low}		Fc γ RIII(CD16)$^+$
LAG-3/CD223$^+$		-
LAP$^+$		IL-18 Rα/IL-1 R5$^+$
LRRC32/GARP$^+$		IL-23 R$^+$
Neuropilin-1$^+$		LAMP-1/CD107a
OX40/TNFRSF4$^+$		NKG2D/CD314$^+$
L-Selectin/CD62L$^+$		NKp30
		NKp44
		NKp46
		S1P1/EDG-1$^+$
		TCRγ/δ$^+$
		-

Indicates Novus Biologicals Antibodies.

Flow Cytometry Antibodies for Helper T Cell Subsets

TH1 CELL MARKERS	TH2 CELL MARKERS	TH9 CELL MARKERS	TH17 CELL MARKERS
CELL SURFACE			
CCR1+	CCR3+	CD3+	CCR4+
CCR5+	CCR4+	CD4+	CCR6+
CD3+	CCR8+	CD8-	CD3+
CD4+	CD3+	CD14-	CD4+
CD8-	CD4+	CD19-	CD8-
CD14-	CD8-	IL-4 Rα+	CD14-
CD19-	CD14-	IL-17 RB+	CD19-
CXCR3+	CD19-	TGF-b RII+	IL-1 RI+
IFN-γ R1/ CD119+	CXCR4+	-	IL-6 Rα+
IFN-γ R2+	IL-4 Rα+	-	IL-21 R+
IL-12 Rβ 2+	IL-17 RB+	-	IL-23 R+
IL-18 Rα/IL-1 R5+	ST2/IL-33 R+	-	TGF-β RII+
IL-27 Rα/ WSX-1/TCCR+	TSLP R+	-	-
INTRACELLULAR			
STAT1+	GATA-3	PU.1+	BATF+
STAT4+	STAT5+	-	RORα+
T-bet/TBX21+	STAT6+	-	RORγt/ RORC2+
-	-	-	STAT3+
SECRETED			
IFN-γ	IL-4	CCL17	IL-17A
IL-2	IL-5	CCL22	IL-17F
TNF-α	IL-9	IL-9	IL-22
Lymphotox-in-α/TNF-β	IL-10	-	IL-26
-	IL-13	-	-

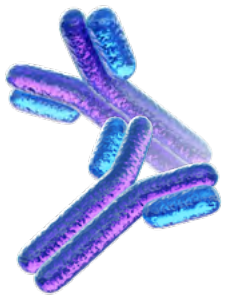
Indicates Novus Biologicals Antibodies.

Flow Cytometry Antibodies For T Cell Activation And Exhaustion Markers

ACTIVATION MARKERS	EXHAUSTION MARKERS
CELL SURFACE	CELL SURFACE
CD25/IL-2 Rα+	2B4/CD244/SLAMF4+
CD38	BTLA/CD272+
CD69	-
HLA-DR	-
Ki67/MKI67+	-
	CD39/ENTPD1+
	CD57low
	CD160+
	CTLA-4+
	CXCR5
	DNAM-1/CD226
	ICOS
	KLRG1low
	LAG-3/CD223+
	NTB-A/SLAMF6
	PD-1+
	TIM-3+
	TIGIT+

Indicates Novus Biologicals Antibodies.

Flow Cytometry Antibodies for immunophenotyping are critical at every step of the T cell therapy manufacturing process. We offer an unparalleled selection of fluorochrome-conjugated antibodies that are validated for flow cytometry. Many of our R&D Systems™ antibodies have been used by HLDA to establish CD nomenclature. Our Custom Antibody Services include development, conjugation, recombinant antibody conversion, and GMP antibodies.

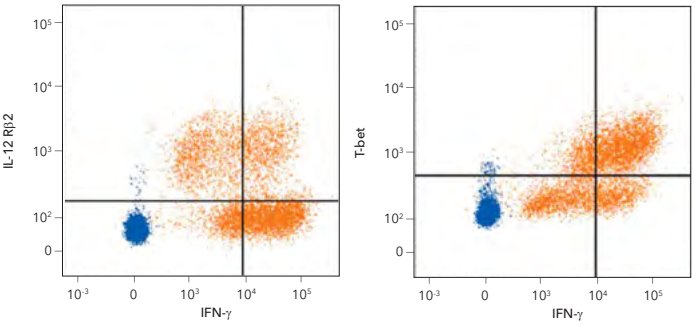


Visit | Flow Cytometry

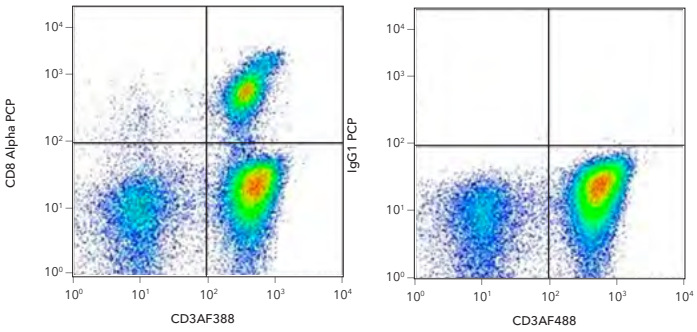
NOVUS BIOLOGICALS™

Our collection of Novus Biologicals flow antibodies includes some of the most highly referenced clones on the market including CD45RA (MEM-56), CD3 (OKT3), and CD103 (Ber-ACT8).

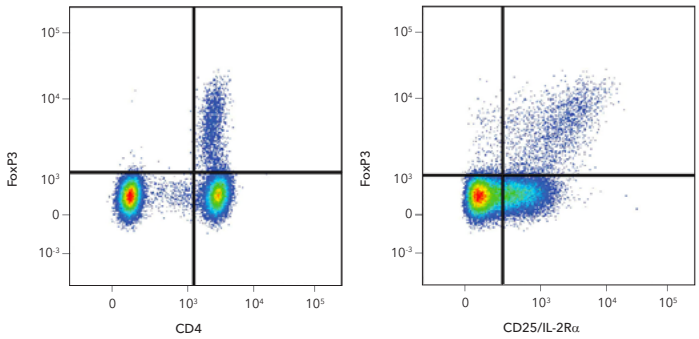
Visit | Solutions from Novus Biologicals



Human Th1 cells were stained for CD4 expression using an Alexa Fluor® 700-Conjugated Mouse Anti-Human CD4 Monoclonal Antibody followed by a PerCP-Conjugated Mouse Anti-Human IFN-γ Monoclonal Antibody, a PE-Conjugated Mouse Anti-Human/Mouse IL-12 Rβ2 Monoclonal Antibody (A), and an Alexa Fluor 488-Conjugated Mouse Anti-Human T-bet Monoclonal Antibody (B). Cells were fixed and permeabilized with the FlowX™ FoxP3 Fixation & Permeabilization Buffer Kit. Flow cytometry quadrants were set based on staining with isotype controls (Catalog # IC003N, # IC006T, # IC002P, # IC0041C, and # IC002G).

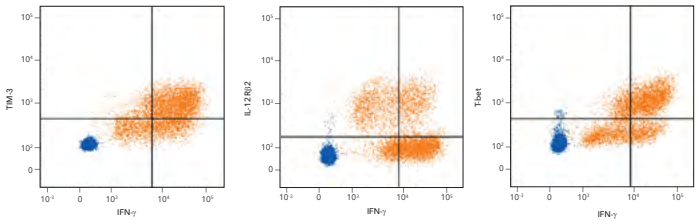


Flow cytometry detection of CD8 on human PBMCs. Cells were stained with either a PerCP-Conjugated Mouse Anti-Human CD8 Monoclonal Antibody (top) or a PerCP-conjugated matched isotype control antibody (bottom), followed by an Alexa Fluor-Conjugated Mouse Anti-Human/Mouse CD3 Monoclonal Antibody.



Flow cytometry detection of FoxP3⁺ regulatory T cells in human PBMCs. Cells were stained with Human CD4 Fluorescein-conjugated Antibody (A) and Human IL-2 Rα/CD25 APC-conjugated Antibody, followed by intracellular staining using Human/Mouse FoxP3 PE-conjugated Antibody (B). To facilitate intracellular staining, cells were treated with the FlowX FoxP3/Transcription Factor Fixation & Perm Buffer Kit.

Multi-Color Flow Cytometry Kits



Th1 cells were generated using the FlowX Human Th1 Cell Multi-Color Flow Cytometry Kit. Cells were stained with the anti-human antibody conjugates included in the kit (CD4 Alexa Fluor 700, TIM-3 Alexa Fluor 594, IL-12 R beta PE, IFN-gamma PerCP, and T-bet/TBX21 Alexa Fluor 488). Dot plots show relative IFN-γ⁺, T-bet⁺, IL-12 Rβ2⁺, and TIM-3⁺ populations in resting CD4⁺ (blue dots, lower left quadrant) and Th1-differentiated cells (orange dots, right quadrants).

FLOW CYTOMETRY RESOURCES

- Flow Cytometry Panel Builder
- Interactive Cell Marker Tool
- Flow Cytometry eHandbook
- Flow Cytometry Training Webinars
- Human Immune Cell Characterization by Flow Cytometry Brochure
- Flow Cytometry Protocols
- Fluorescent Probes and Dyes Brochure

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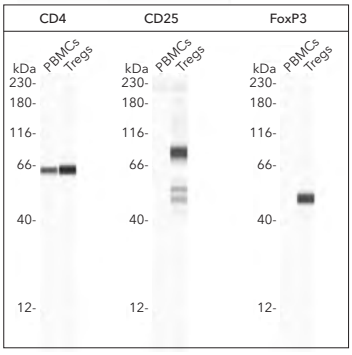
Browse | Simple Plex Automated ELISAs

Simple Western™ assays are fully automated, capillary-based immunoassays that offer picogram-level sensitivity for cell characterization. Simple Western assays combine the power of CE-SDS or cIEF with the sensitivity of immunodetection, enabling size- and charge-based screening of complex sample types.

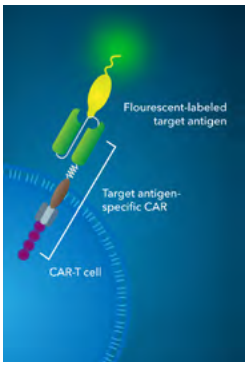
Compare | Simple Western Systems



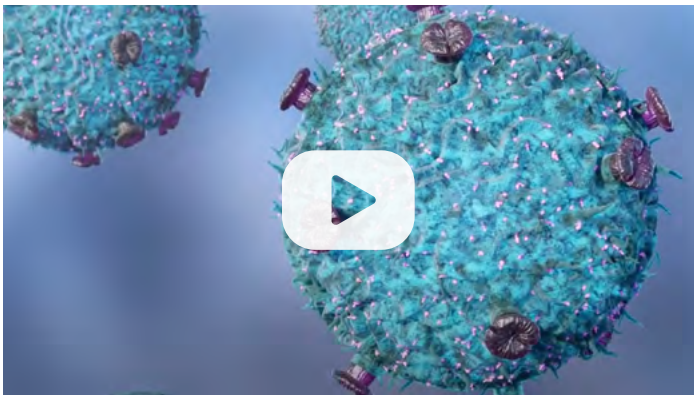
Characterization of regulatory T cells on Simple Western. Cell sample lysates were for analyzed for FoxP3, CD25, and CD4. FoxP3 and CD25 are expressed byTregs but not by PBMCs.

RESOURCES

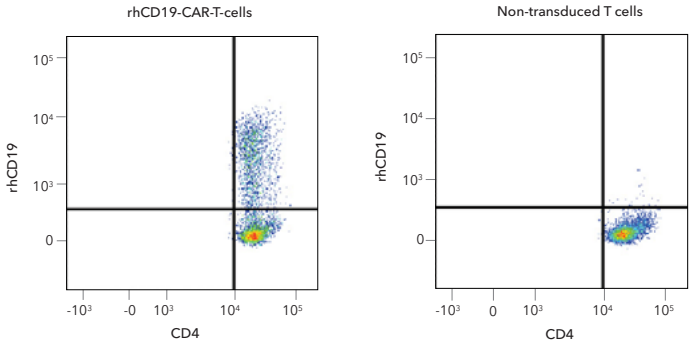
Western Blot eHandbook



Fluorokine™ Fluorescent-Labeled Proteins are valuable for directly evaluating the expression of chimeric antigen receptors (CAR) on your engineered CAR-T cells. Fluorescent-labeled recombinant proteins allow flow cytometry analysis of cells expressing the corresponding CAR. We offer an expanding selection of fluorescent-labeled recombinant proteins including BCMA, CD19, Siglec-2/CD22, and Siglec-3/CD33.

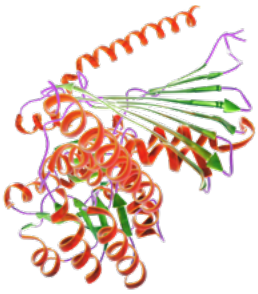


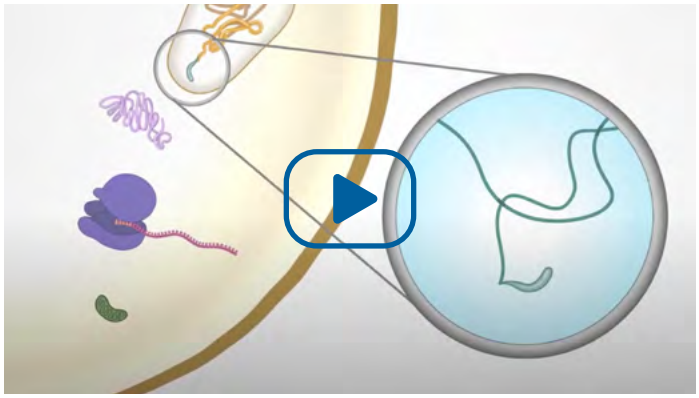
Browse | Fluorescent-Labeled Proteins



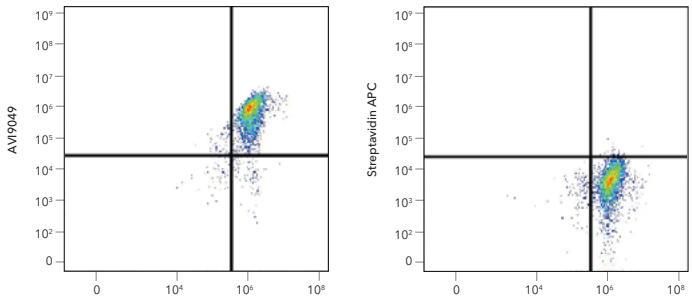
$CD4^+CD8^+$ T cells were transduced with a human CD19-CAR construct (left) or not transduced (right) and then cultured for 11 days. Cells were stained with a human-CD4 PE-Cy7-conjugated antibody and Recombinant Human CD19 Fc Chimera Atto 488 Protein and analyzed by flow cytometry.

Avi-tag Biotinylated Proteins enable the indirect phenotypic analysis of engineered T cells. These proteins are specifically biotinylated at a single site to ensure consistent, uniform biotinylation. We offer a wide selection of Avi-tag biotinylated recombinant proteins for detection of immune checkpoint proteins on T cells. A secondary step with fluorochrome-labeled streptavidin enables detection by flow cytometry.



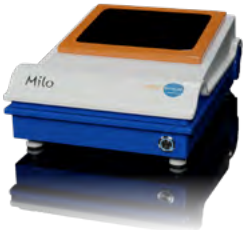


Explore our selection of Avi-Tag Biotinylated Proteins.
Download Avi-tag Biotinylated Proteins Application Note.

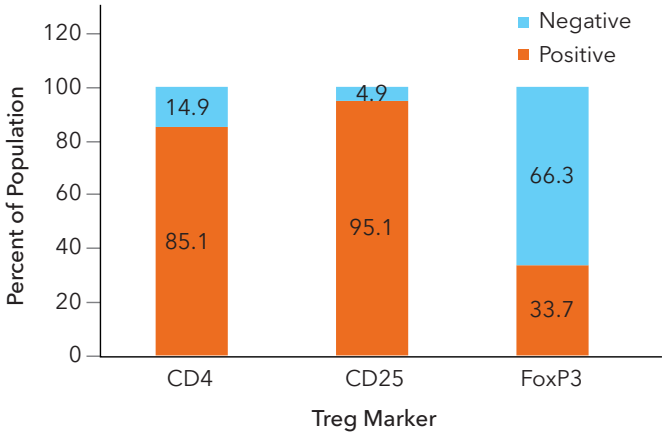


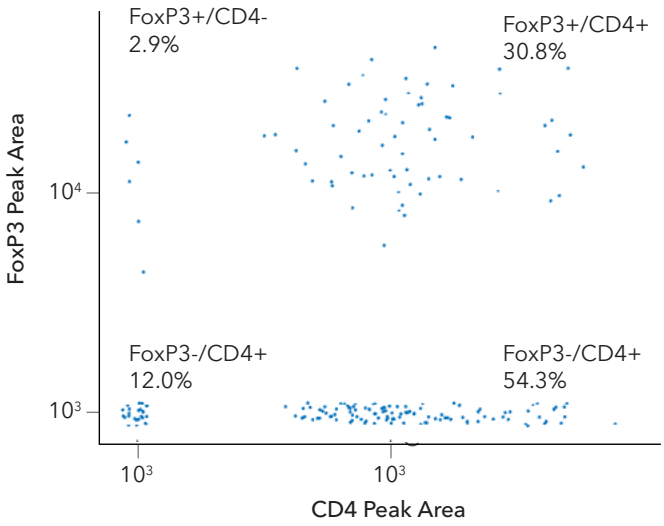
In a functional flow cytometry test, Recombinant Human PD-L1/B7-H1 His-tag Avi-tag Protein binds to HEK293 human embryonic kidney cell line transfected with recombinant human PD-1 and EGFP (A). Ligand binding was detected by staining cells with APC-conjugated Streptavidin which does not stain the cells in the absence of recombinant protein (B).

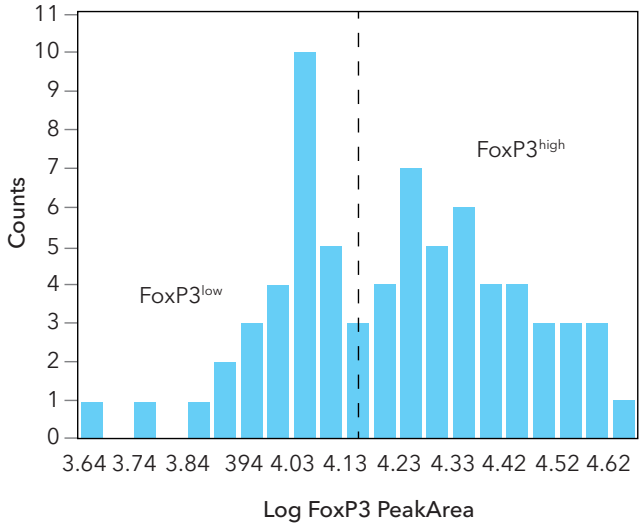
Single-Cell Western profiles cell product heterogeneity by measuring protein expression in thousands of single cells in a single run, up to 12 proteins per cell using a variety of multiplexing strategies.



Learn About | Single-Cell Western







Single-Cell Western characterization of regulatory T cell subpopulations. Single cells were resolved and analyzed with Human CD4 Antibody, Human CD25/IL-2R alpha Antibody, and Human FoxP3 Biotinylated Antibody (A). Two-dimensional scatter plot of the subpopulations present based on the presence or absence of FoxP3 and CD4 (B). Histogram showing classification of Treg populations based on intracellular FoxP3 expression (C).

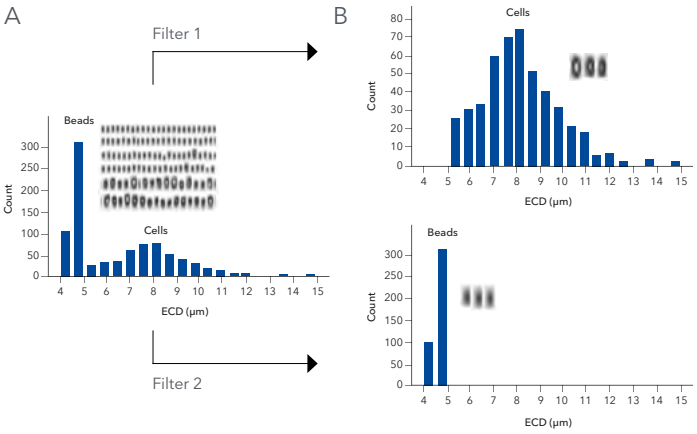
exosomedx™
a bio-techne® brand

Exosome analysis for RNA, DNA, and protein components provides a cellular signature for the functionality and activity of your T cells. Longitudinal analysis of culture fluid exosomes for gene expression or proteomic profiles can serve as a complementary and orthogonal readout to immunoassay data.

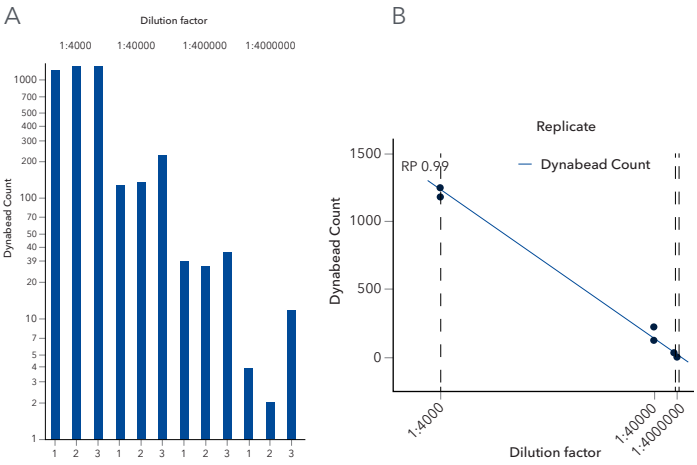
Visit | Exosome Diagnostics

Micro-Flow Imaging™ (MFI) harnesses the power of digital microscopy and microfluidics with 21 CFR Part 11-compliant direct particle imaging. MFI provides quantitative, accurate, and reproducible characterization of subvisible particles. MFI View System Suite and Image Analysis software enables the creation of powerful filters for quantitating particle number and morphology.

Learn About | Micro-Flow Imaging



Distinguish between particle types in multiple tandem samples with MFI. (A) MFI Image Analysis software filters were set for Jurkat T cells and Dynabeads, where ECD is the equivalent circular diameter and Intensity Std is the standard deviation of the intensity of all pixels of that particle. (B). MFI distinguishes between the two particle types within a mixed population.



The reproducibility of MFI is shown by a ten-fold dilution series of Jurkat T cells and Dynabeads. For each dilution, replicate samples showed a high degree of consistency (A), with values as low as 5-10 beads/mL detected. Panel B shows the linearity across this dilution series, with an R^2 value of ≥0.99.

MFI RESOURCES

Cell Contaminant Screening

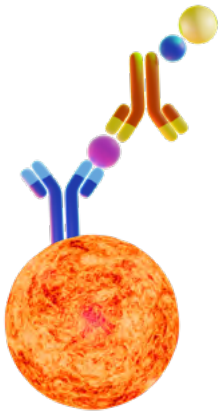
Automation of Particle Analysis

MFI Image Analysis Software

Assess the Purity of Your Cell Therapy with Micro-Flow Imaging

Determining Residual Bead Count In CAR-T Cell Manufacturing

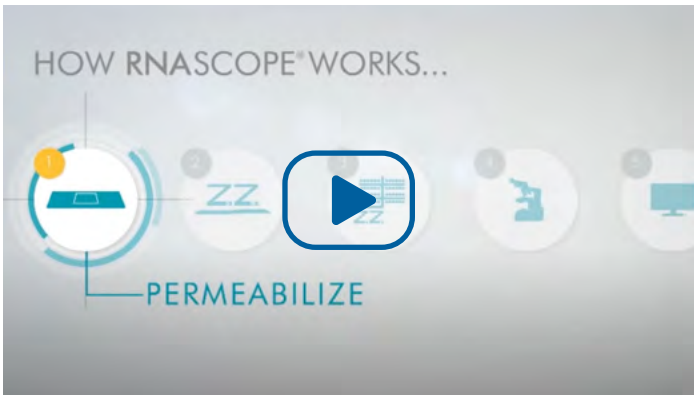
5 | MONITORING EFFICACY AND TOXICITY



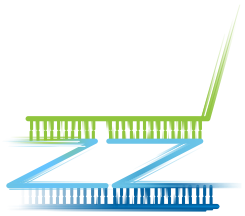
The development of adoptive T cell therapies is advancing rapidly but still has considerable room for improvements in performance and safety. To this end, it is necessary to understand how T cell therapies perform after administration, both in preclinical animal models as well as in biopsy samples from treated patients.

See the Analytics Performance chapter for more information.

Monitoring T Cell Therapies After Administration
Analyzing the integrity and trafficking of your engineered T cell therapy in situ provides detailed information about infiltration and persistence in the tumor.

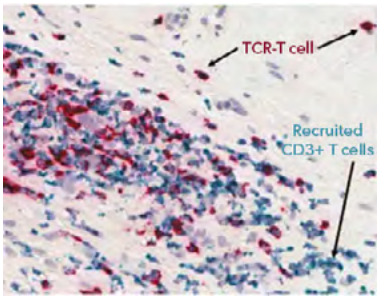


RNAscope™ ISH Assays for tissue biopsy analysis utilize a novel multiplex technology with a patented probe design that amplifies target-specific signals but not background noise, delivering clear and actionable results with high sensitivity and specificity.



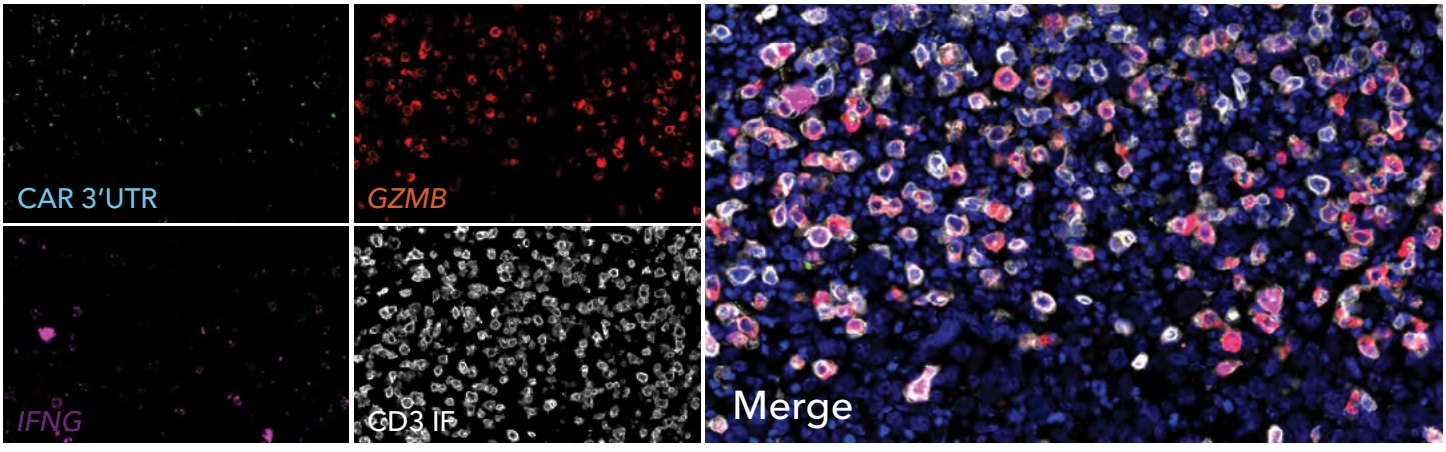
Browse | RNAscope ISH Assays

RNAscope Duplex Assay analysis of NY-ESO-1 TCR-T cells infiltrating a post-treatment liposarcoma patient biopsy. TCR-T cells and local recruitment of CD3⁺ T cells were observed with TCR UTR (red) and CD3ε (green) probes.



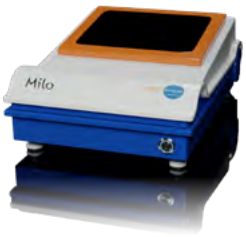
SPATIAL BIOLOGY RESOURCES

CAR-T Cell Target Safety, Biodistribution, and Tumor Infiltration Analysis with RNAscope Application Note



Trafficking of activated CAR-T cells to the tumor site. The RNAscope™ LS Multiplex Fluorescent Assay was combined with immunofluorescence to visualize tumor infiltration by activated anti-BCMA CAR-T cells. RNAscope ISH for the 3′ UTR of the CAR vector (green), Granzyme B (red), and IFN-gamma (pink) was followed by CD3 immunofluorescence (white) in xenograft tumors from RPMI-8226 mice treated with anti-BCMA CAR-T cells.

Exosomes carry RNA, protein, and DNA and allow multi-parameter dynamic and real-time monitoring of T cell status and host responses. Our Exosome Diagnostics brand has a patented and proven process for the isolation of exosomes from cell media and other biofluids.



Visit | Exosome Diagnostics

Monitoring the Host Response

Monitor inflammatory markers of cytokine release syndrome, infiltration of host immune cells into the tumor, the development of local tissue responses, and biomarkers of disease progression.

Browse | Immunoassays for Immune Response Profiling

Watch | Monitoring Host Immune Responses Webinar

Immunoassay Formats for Select Disease Biomarkers

BIOMARKER	ELISA	SIMPLE PLEX	LUMINEX	ANTIBODIES	PROTEOME PROFILER	BIOSPACIFIC
Adiponectin/ Acrp30	Yes	Yes	Yes	Yes	Yes	Yes
Alpha-Fetoprotein/ AFP	Yes	Yes	Yes	Yes	Yes	Yes
Apolipoprotein A1	Yes	-	-	Yes	Yes	Yes
CA125/MUC16	Yes	Yes	Yes	Yes	Yes	Yes
CD25/IL-2 Rα	Yes	Yes	Yes	Yes	Yes	-
CD31/PECAM-1	Yes	-	Yes	Yes	Yes	-
CD117/c-kit	Yes	-	Yes	Yes	Yes	-
Chitinase 3-like 1	Yes	Yes	Yes	Yes	Yes	-
Clusterin	Yes	Yes	Yes	Yes	Yes	-
CXCL9/MIG	Yes	Yes	Yes	Yes	Yes	-
Dkk-1	Yes	Yes	Yes	Yes	Yes	-
EGFR	Yes	-	Yes	Yes	Yes	Yes
Enolase 2	Yes	-	Yes	Yes	Yes	-
ErbB2/Her2	Yes	Yes	Yes	Yes	Yes	-
FABP4	Yes	-	Yes	Yes	-	Yes
Fetuin A	Yes	-	Yes	Yes	Yes	-
FGF basic/FGF2	Yes	-	Yes	Yes	Yes	-
Kallikrein 3/PSA	Yes	-	Yes	Yes	Yes	Yes
Lipocalin-2/NGAL	Yes	Yes	Yes	Yes	Yes	Yes
MMP-9	Yes	Yes	Yes	Yes	Yes	-
PCSK9	Yes	Yes	Yes	Yes	Yes	-
Progranulin	Yes	-	Yes	Yes	Yes	-
Resistin	Yes	Yes	Yes	Yes	Yes	-
RBP4	Yes	-	Yes	Yes	Yes	-
S100A8/S100A9	Yes	-	-	Yes	-	Yes
Serpin E1/PAI-1	Yes	Yes	Yes	Yes	Yes	-
uPA/Urokinase	Yes	Yes	Yes	Yes	Yes	-
VAP-1	Yes	-	Yes	Yes	Yes	-

biospacific™
a bio-techne® brand

Our BiosPacific brand offers a wide range of monoclonal and polyclonal antibodies along with recombinant and native proteins for both routine and niche cancer markers. BiosPacific supplies multiple clones for markers such as AFP, CA-125, CA19-9, CA15-3, Pepsinogen I & II, PSA and HE4 which are designed and developed with the commercial diagnostic market in mind.

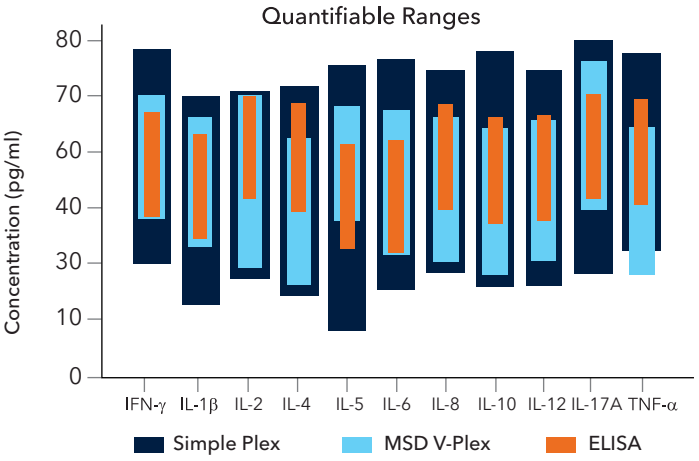
Visit | BiosPacific

Unique Animal Model Systems - During preclinical cell therapy testing, evaluate your animal model with our extensive offering of immunoassays for multiple species. Monitor host responses with assays developed and standardized with the exact species of protein you need to measure.

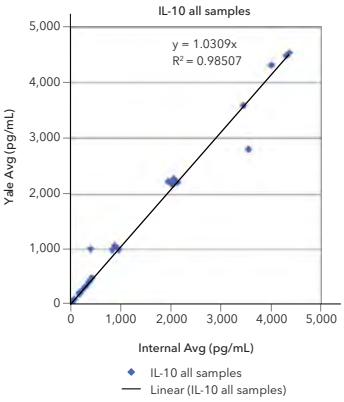
Browse | Immunoasssays for Unique Animal Model Systems

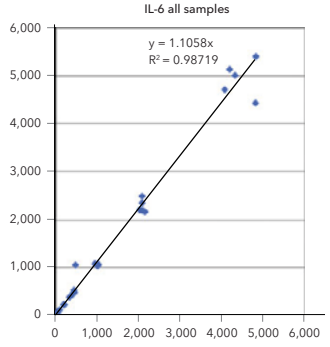
Simple Plex™ Assays are fully automated immunoassays that offer picogram sensitivity, up to 4 logs of dynamic range, and results in 90 minutes or less. Simple Plex assays minimize hands-on time with built in automation that enables consistent data across multiple users and site locations. Microfluidics cartridges run samples in individual channels to eliminate cross-reactivity and are available in single-plex and multi-analyte configurations.

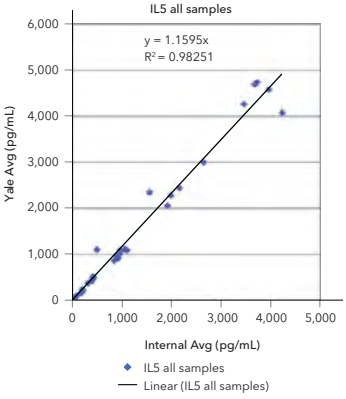
Browse | Simple Plex Automated ELISAs



Dynamic range of Simple Plex compared to ELISA. Simple Plex offers greater dynamic range of up to 4 logs for a broad range of cytokines compared to plate-based ELISA and other technologies.

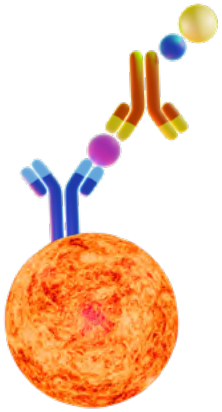




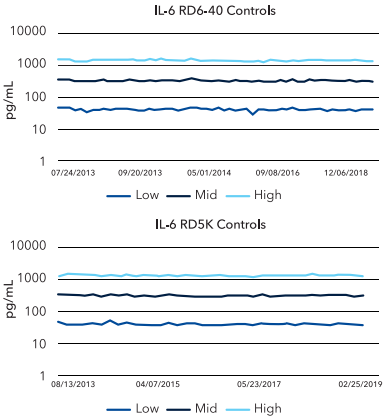


Sample Reproducibility of Simple Plex Assays: Standard curves for IL-5, IL-6, and IL-10 were generated by two separate laboratories, at different times, using the same batch of panels. Reproduced from Aldo, P. et al. Am. J. Reprod. Immunol. 75:678.

Luminex® assays enable multiplex cytokine profiling to monitor cytokine release syndrome (CRS). These assays are available as off-the-shelf, curated panels or custom panels built from our selection of over 450 target analytes. These assays maximize multiplexing capacity and flexibility while maintaining target specificity. Luminex profiling of up to 50 analytes per sample increases efficiency and improves cost-effectiveness, available for human, non-human primate, mouse, rat, and porcine systems.



Browse | Luminex Assays



The Human IL-6 XL Magnetic Performance Assay maintains lot-to-lot consistency over the long term. Levey-Jennings control plots show IL-6 lot-to-lot consistency in RD5K diluent (A) and RD6-40 diluent (B) over 6 years.

ANALYTE	INTRA-ASSAY (%CV)	INTER-ASSAY (%CV)
BDNF	8.16	13.3
CCL2/MCP-2	3.02	10.5
CCL5/RANTES	3.72	17.0
CCL11/Eotaxin	7.98	15.4
CCL20/MIP-3α	8.41	17.3
CD40 Ligand	9.30	15.0
CXCL2/GROβ	7.76	13.1
CXCL10/IP-10	2.95	12.2
CXCL11/I-TAC	6.23	13.7
CXCL13/BLC	5.79	12.5
FGF basic	5.60	13.1
G-CSF	5.55	14.2

ANALYTE	INTRA-ASSAY (%CV)	INTER-ASSAY (%CV)
GM-CSF	7.11	14.1
Granzyme B	9.75	18.6
INF-α	5.17	12.4
INF-β	10.9	15.2
INF-γ	6.36	13.0
IL-1β	2.55	12.7
IL-10	8.55	14.1
IL-12 p70	4.92	17.1
IL-13	7.97	17.5
IL-15	4.99	18.2
IL-17A	4.38	19.0
IL-2	5.32	18.1
IL-21	5.84	19.0
IL-4	4.92	17.5
IL-5	4.14	16.5
IL-6	6.80	17.8
IL-7	6.57	17.7
IL-8/CXCL8	6.87	17.5
PDGF-AA	6.52	25.0
PDGF-BB	3.37	16.9
PD-L1	8.36	19.4
TGF-α	5.43	18.7
TNF-α	3.68	17.2
VEGF	4.79	18.4

Precision is key for confidence in your data. Data from the Non-Human Primate XL Cytokine panel indicate that all analytes have an intra-assay CV below 11% from 40 reportable results and an inter-assay CV below 26% across 31 assays.

LUMINEX RESOURCES

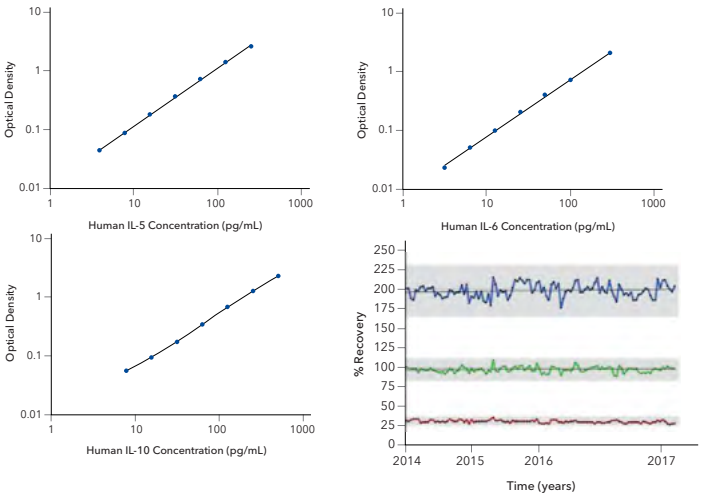
Luminex Custom Assay Tool

Luminex Troubleshooting Guide

Quantikine ELISAs and Quantikine High Sensitivity ELISAs are fully validated and optimized immunoassays that undergo a rigorous quality control process to ensure lot-to-lot consistency. They are built with in-house components to guarantee unparalleled control over critical elements that affect results and performance over time. Quantikine QuicKit ELISAs maintain the high quality of Quantikine but deliver results in 90 minutes with a simplified workflow. DuoSet ELISAs are a flexible and economical assay development alternative that is adaptable across multiple platforms in 19 different species.

Browse our selection of ELISA kits in each format.

Quantikine ELISAs are consistent over the long term. Quantitation of Human IL-6 in High, Medium, and Low Controls. High (blue line), medium (red line), and low (green line) controls are assayed with every manufactured lot of the Human IL-6 Quantikine ELISA Kit. Control values fall within acceptable ranges (gray bars) and remain consistent from lot to lot.



ELISA RESOURCES

The ELISA Guide

Custom ELISA Services

Quantikine ELISA Validation

Avoid False Positive Data Application Note

DuoSet ELISA Development Systems

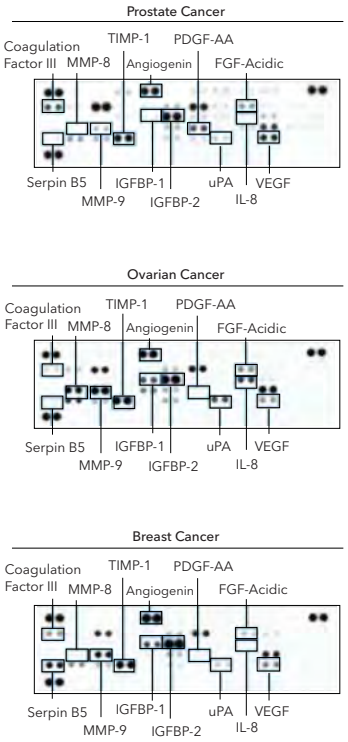
Quantikine QuicKit ELISAs

Proteome Profiler™ Antibody Arrays are high throughput, cost-effective tools for early-stage analyte profiling. They deliver clear and consistent data with superior specificity, low background noise, and no cross-reactivity. Arrays are based on nitrocellulose membranes that are pre-spotted in duplicate with carefully screened, high-quality capture antibodies for multiplexing. Arrays are available for detection of either intracellular or secreted analytes in human, mouse, and rat and do not require specialized equipment.

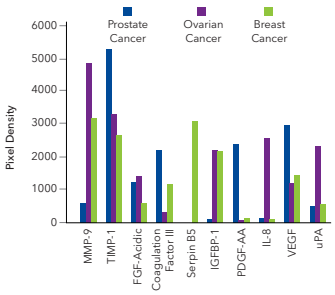
Browse | Proteome Profiler Antibody Arrays

Monitoring Angiogenesis

A



B



Analysis of angiogenesis-related proteins in tissues from human prostate, ovarian, and breast cancers with the Proteome Profiler Human Angiogenesis Array Kit (A). Histogram profiles for select analytes were generated by quantifying the mean spot pixel densities from the array membrane using image software (B).