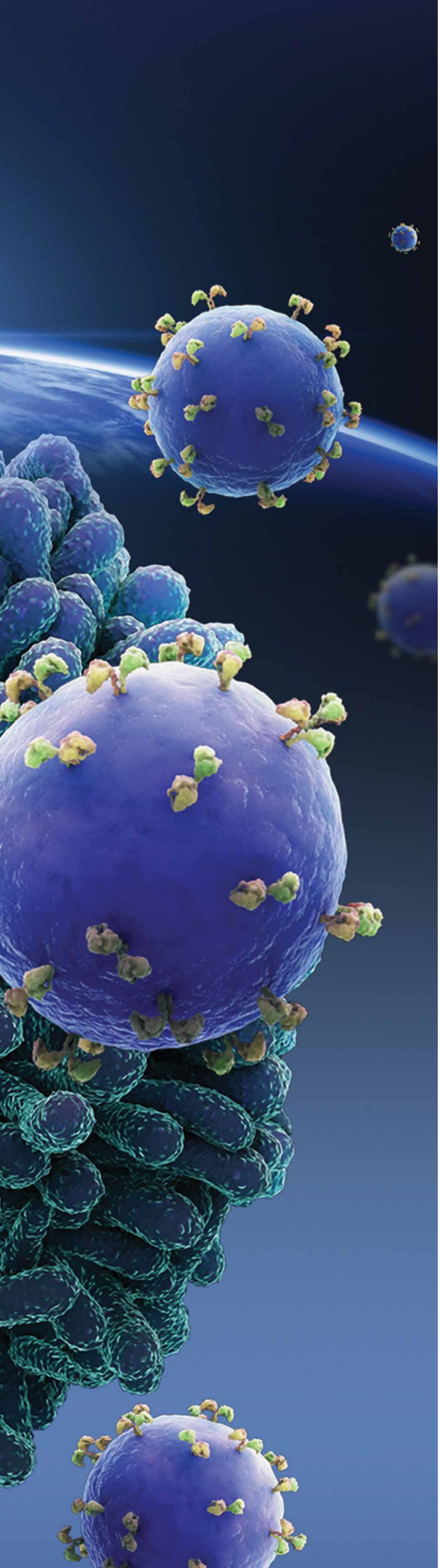
Cancer research

# Immuno-oncology product resource guide

## Your comprehensive guide to tools for immuno-oncology research

**Our goal** is to support your **immuno-oncology research** with a comprehensive range of tools and technologies that maximize your time, budget, and data—helping to accelerate your path to discovery and translation to a clinical application.

In this guidebook, you will discover educational resources and solutions for a number of immuno-oncology research approaches, including checkpoint inhibition, CAR T cell therapy, and cancer vaccine research. Learn about our capabilities, from leveraging innovative products and techniques to time-saving workflow applications. We're committed to partnering with you for your next breakthrough.



# Contents

# Immuno-oncology review

## What is I-O?

Immuno-oncology (I-O), also known as cancer immunotherapy, is a rapidly growing field that studies the ways in which the body's own immune system can fight cancer.

## Why does I-O research matter?

I-O research aims to develop cancer immunotherapies that go beyond traditional methods such as surgery, chemotherapy, and radiation, by enabling the adaptive immune system to recognize and specifically attack cancer cells while leaving healthy ones undamaged. I-O research can potentially uncover ways to enable immunogenicity of all types of cancer and facilitate long-lasting, protective immunity against future recurrence [1-3]. Recent breakthroughs in checkpoint inhibition, chimeric antigen receptor (CAR) T cell therapy, and cancer vaccines illuminate the full capabilities of the immune system and how it may be harnessed to combat cancer.

While every immune cell has a key part to play in the landscape of I-O, T cells and T cell–mediated responses are the focal points of I-O research today.

## What are some of the promising areas in I-O research?

This handbook will provide an overview of three currently trending I-O research areas: checkpoint inhibition, CAR T cell therapy, and cancer vaccines. Figure 1 (adapted from Chen and Mellman [4]) shows where they correlate within the cancer-immunity cycle.

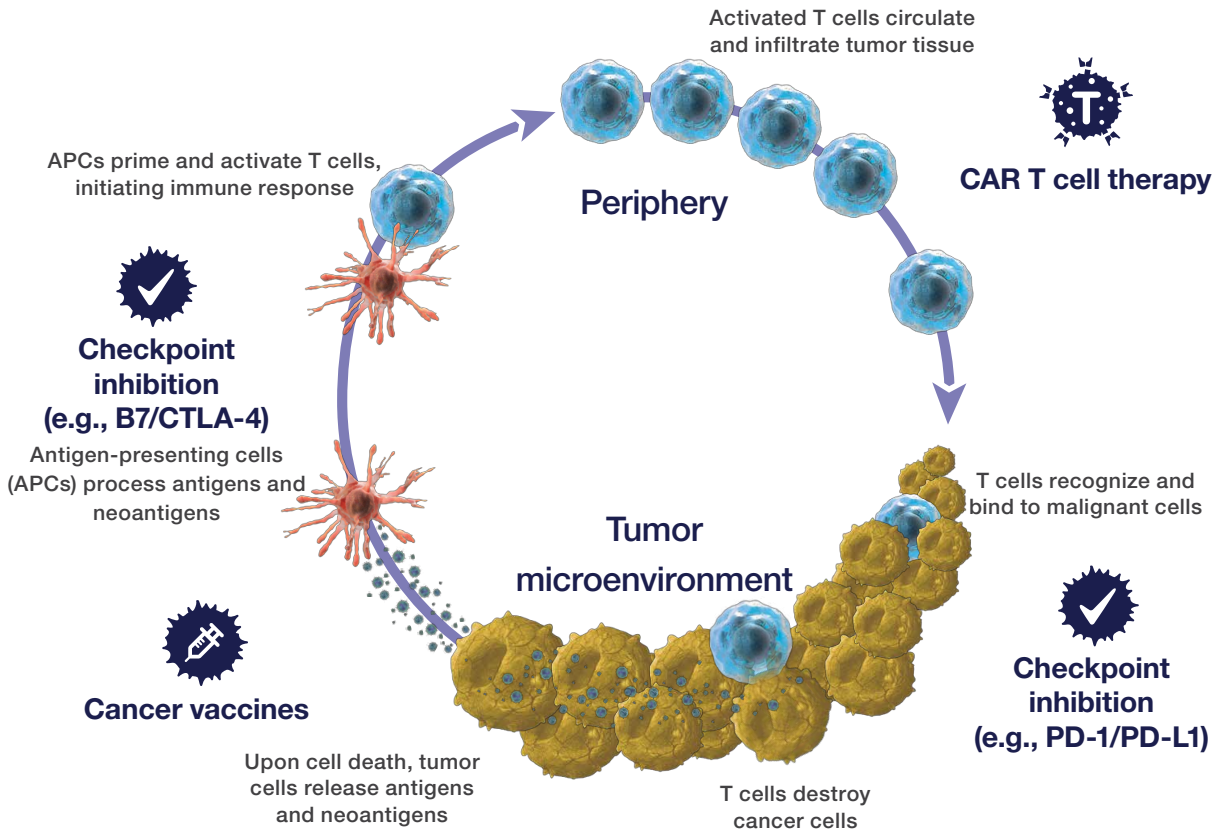



**Figure 1. Stages of the adapted cancer-immunity cycle [4] can be impacted by I-O approaches such as checkpoint inhibition, CAR T cell therapy, and cancer vaccines, as indicated by the icons.**

## Checkpoint inhibition

Immune checkpoints are cell pathways crucial in maintaining a normal immune response and protecting tissues from damage when the immune system is activated [5,6]. Cancer cells dysregulate immune checkpoints and use them as a mechanism of immune resistance. Understanding the interactions between tumor and immune cells is one of the main approaches in I-O research [7,8].

There are natural mechanisms in place that serve to regulate T cell activity via interactions with the T cell receptor (TCR). For example, PD-1/PD-L1 is a coinhibitory pathway that "masks" cancer cells from T cell recognition, thereby preventing the attack by T cells. Antibodies that target the PD-1/PD-L1 pathway and bind to PD-1 suppress its coinhibitory function. The T cells then recognize the cancer cells and cytotoxic activity commences.

Another example of T cell regulation is the B7/CTLA-4 pathway that plays a role during the priming of a T cell by an antigen-presenting cell (APC). Blocking of the CTLA-4 receptor by an antibody allows T cell activation, resulting in an anticancer immune response.

There are multiple costimulatory and coinhibitory receptor–ligand interactions between APCs and T cells. For T cell activation or suppression, T cells must recognize their cognate antigens through TCRs and then respond to costimulatory (for activation) or coinhibitory (for suppression) receptor–ligand interactions, examples of which are shown in Figure 2 [6,9].

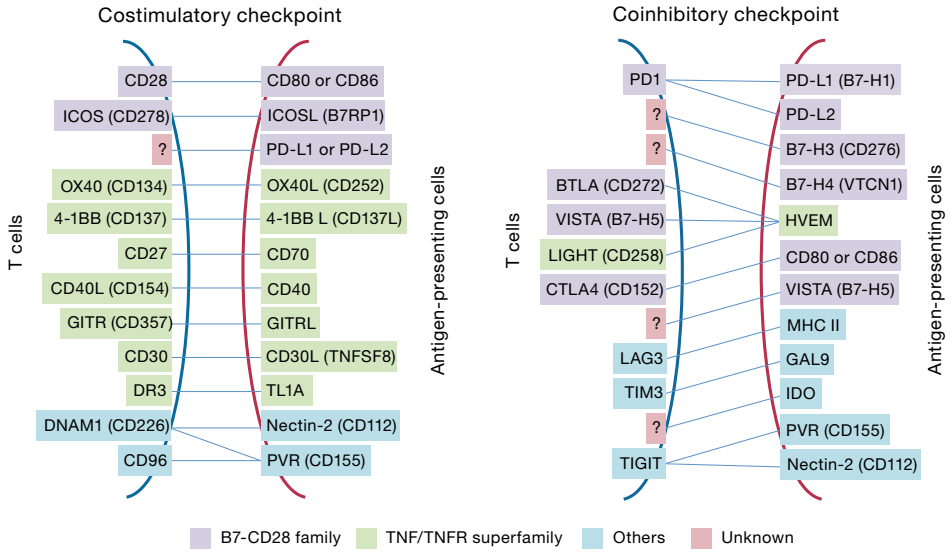



**Figure 2. Multiple costimulatory and coinhibitory receptor–ligand interactions between APCs and T cells.** One important family of membrane-bound molecules that bind both costimulatory and coinhibitory receptors is the B7-CD28 family (purple boxes); all of the B7 family members and their known ligands belong to the immunoglobulin superfamily. Another major category of signals arises from tumor necrosis factor (TNF) family members (green boxes), which regulate the activation of T cells in response to cytokines.

## Adoptive cell therapy (ACT) and CAR T cell therapy

ACT targets the immune system, enabling the body's natural ability to fight the cancer, instead of directly targeting the cancer itself. This is accomplished by genetically modifying a subject's own T cells to target antigens selectively expressed on cancer cells [10,11]. Successful applications of ACT include:

- Tumor-infiltrating lymphocytes (TILs) are taken from tumor tissue, modified *ex vivo*, and infused in activated form back into the body to re-infiltrate the tumor and attack tumor cells.
- CAR T cells are generated from the body's own T cells and are engineered to express antibody-like chimeric antigen receptors for targeting specific cancer cells via surface proteins or intracellular proteins, inducing anticancer attack (Figure 3).

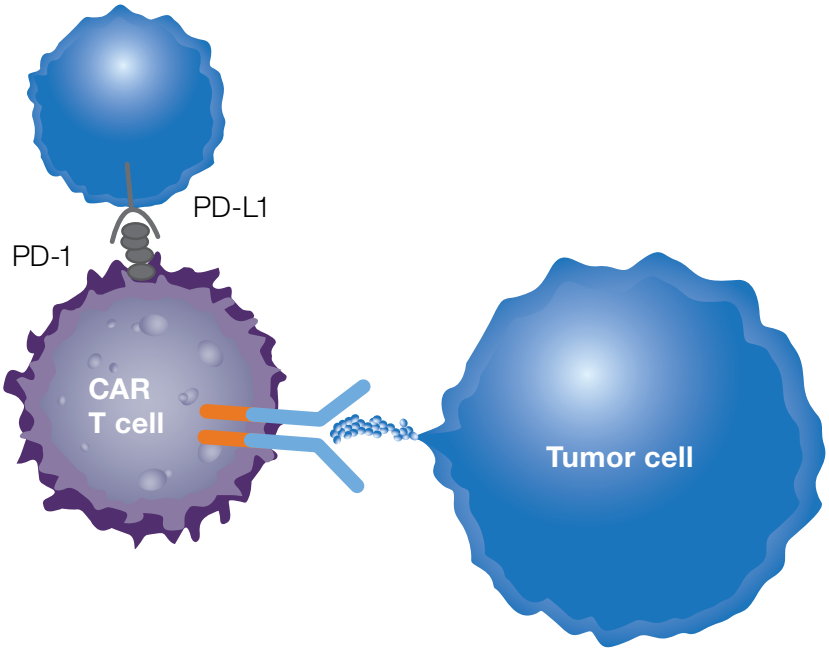



**Figure 3. CAR T cell engineering involves genetically modifying an individual's own T cells to target antigens selectively expressed on cancer cells.**

## A closer look: CAR T cells

The TCR participates in the activation of T cells [2]. Its stimulation is triggered in response to cells expressing major histocompatibility complex (MHC) molecules with an antigen. Tumor-specific TCRs can be genetically engineered to recognize specific cancer cell populations. TCR technology is unique as it recognizes both intracellular and cell surface proteins, conferring a broad array of antigen targets. Limitations include patient-specific human leukocyte antigen (HLA) restrictions and the lack of unique tumor-specific antigens.

CARs are fusion proteins combining intracellular T cell components and extracellular antigen-recognition domains from a monoclonal antibody [2,10,11]. They can be constructed by linking the variable regions of the heavy and light chains of the antibody to intracellular signaling chains (such as CD3-zeta, CD28, and 4-1BB) or other signaling factors. T cells that are engineered to express CARs are not limited by HLAs, since a CAR molecule recognizes an intact cell antigen on the surface of a cancer cell. However, they are limited by their inability to recognize mutated intracellular proteins.

## Cancer vaccines

Vaccines represent another important I-O research area aimed at enabling the immune system to recognize cancer as a threat. This method is antigen-based, relying on the ability of the immune system to recognize the protein to induce the immune response. Scientists endeavor to identify new tumor-associated antigens, called neoantigens, released within the tumor microenvironment [12]. This aids in understanding how tumors form and spread, which informs the development of vaccines. Another method called dendritic cell (DC) therapy utilizes tumor fragments to activate extracted DCs. This activation turns DCs into APCs, which are then infused back into the subject to induce a secondary immune response, including antibody production [12]. Finally, new combination therapies and personalization methods are also being studied to further enrich the capabilities of anticancer immunity.

## General I-O workflow

Thermo Fisher Scientific offers many research platforms and products to help you better understand the interplay between the immune system and cancer. Expand experimental capabilities with our instruments, assays, and reagents to accelerate the development of the cancer immunotherapies of tomorrow. In the following chapters, explore our solutions by approach (Figure 4).

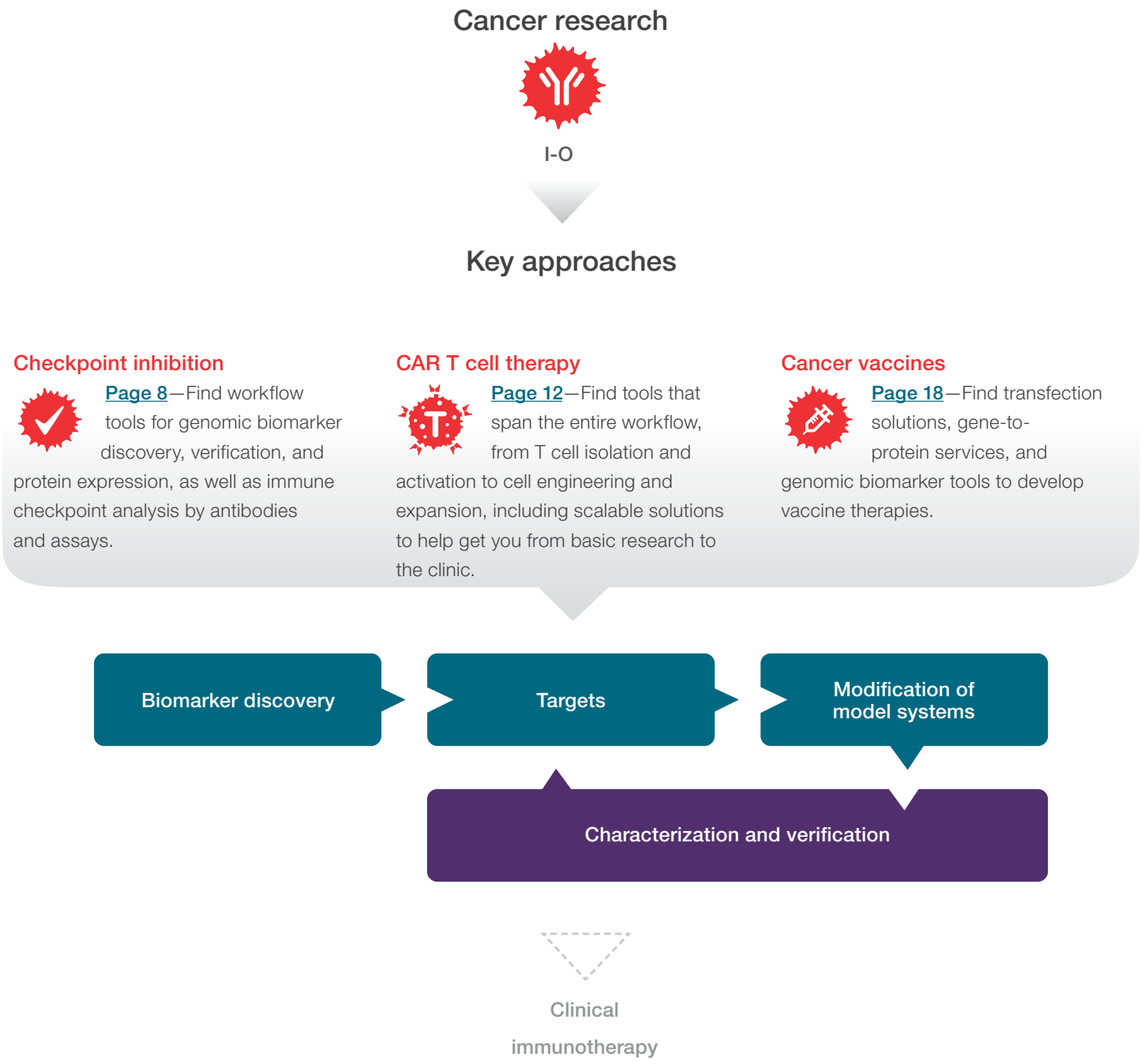
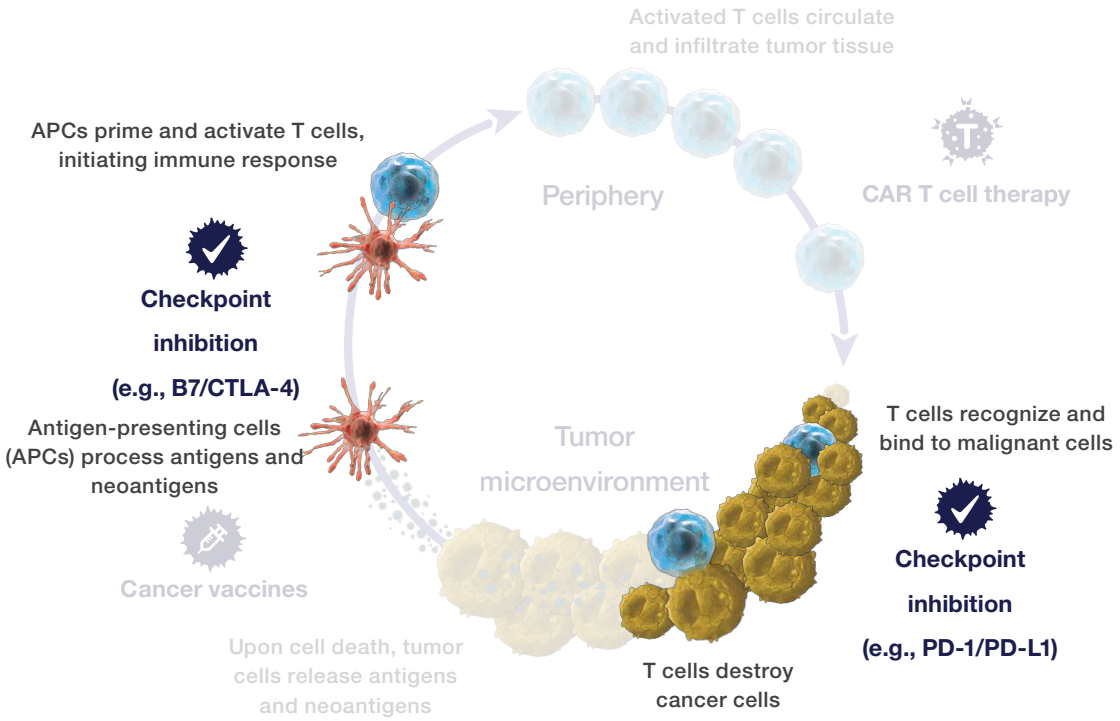



**Figure 4. A growing focal area within cancer research, I-O encompasses a robust workflow.** This starts with the biomarker discovery phase, continues into further research on targets of interest, including contextual studies within model systems, and finally may conclude with characterization and verification. This workflow is applicable across different approaches within I-O, including checkpoint inhibition, CAR T cell therapy, and cancer vaccine research.

# Solutions for checkpoint inhibition

Identifying and validating predictive biomarkers for checkpoint immunotherapy is important to optimize therapeutic benefit, minimize toxicity risk, and guide combination therapy approaches. Discover a wide variety of solutions, from genomic biomarker discovery and verification tools to protein biology and cell analysis products, that can help you better discern stratification of responders and nonresponders to checkpoint immunotherapy.




## Genomic biomarker discovery and verification tools

- **Applied Biosystems™ Clariom™ D Pico assays**—Get a deep view of the transcriptome to rapidly discover novel biomarkers. Analyze coding and long noncoding RNA as well as alternative splicing events from as little as 100 pg of total RNA with this microarray-based solution.
- **Applied Biosystems™ Clariom™ S Pico assays**—Discover gene-level analysis of well-annotated genes across the transcriptome from as little as 100 pg of total RNA. Quickly identify important gene-level signatures and pathways and screen large numbers of samples with microarray-based high-throughput, automated formats.
- **Applied Biosystems™ TaqMan™ Array Human Immune Response Plate**—Utilize a 96-well plate for quantitative gene expression analysis. Accurately analyze genes from 9 classes of immune system functions, including cell surface receptors, transcription factors, cytokines and cytokine receptors, and cell cycle and protein kinases.
- **Applied Biosystems™ TaqMan™ Array Human Immune Panel**—Get quantitative gene expression analysis of a comprehensive set of gene targets related to the human immune response. The easy-to-use 384-well microfluidic card includes targets for immune regulators, apoptosis markers, ischemia markers, and more.
- **Applied Biosystems™ TaqMan™ Gene Expression Assays**—Use the gold standard in real-time PCR gene expression analysis that provides a fast, simple method for verification of gene expression biomarkers.
- **Ion Torrent™ Oncomine™ Immune Response Research Assay**—Explore a targeted next-generation sequencing gene expression assay designed to measure the expression of genes involved in tumor–immune system interactions and identify biomarkers for immunotherapy.

Find out more at [thermofisher.com/checkpointinhibitor](thermofisher.com/checkpointinhibitor)

## Straight from the scientist

On transcriptomics analysis using Clariom assays to identify biomarkers and drug resistance mechanisms: “I am a molecular biologist by training, and I can easily use this technology any time. I can go back to the software myself and further analyze other genes that are downstream.”

Yesim Gökmen-Polar, PhD
Assistant Research Professor, Department of Pathology and Laboratory Medicine, Indiana University
thermofisher.com/drugresistance

## Did you know?

We have other protein expression systems like the Gibco™ ExpiSf™ Expression System, the first-ever chemically defined baculovirus expression system that can generate up to 3x more protein compared to other insect expression platforms.
thermofisher.com/expisf

### Protein expression

- **Gibco™ Expi293™ Expression System**—Leverage a rapid, high-yield system that allows access to proteins derived from recombinant 293 cells in just 5 to 7 days; designed to deliver up to 6x more protein production in just one week, compared with other transient 293 systems that can take 2 weeks or more.
- **Gibco™ ExpiCHO™ Expression System**—Experience higher protein yields compared to alternative transient systems, saving you precious time, incubator space, and plasticware costs. Now you can express the same amount of protein in a single flask that other transient CHO systems express in 20 or more flasks. The system also provides multiple protocols to fit your workflow, and it can be scaled up or down based on your needs for greater throughput early in discovery, or higher yields as you focus on selected proteins (Figure 5).

Need to analyze your protein? See our solutions for protein analysis on page 25.

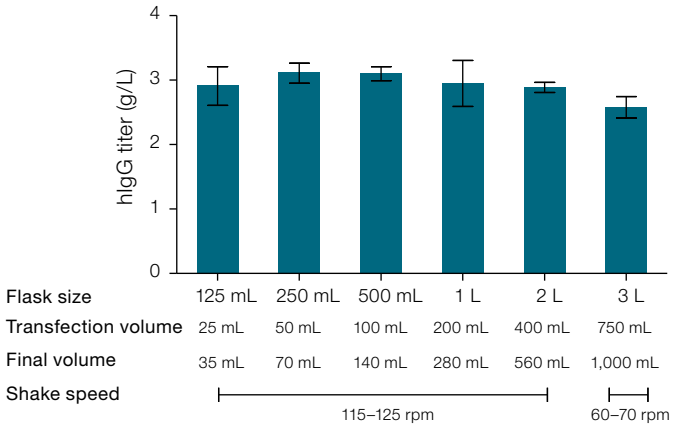



**Figure 5. Scalability of the ExpiCHO system: directly scalable from 125 mL to 2 L flask sizes; 3 L flasks require reduction of shake speed to 70 rpm.**

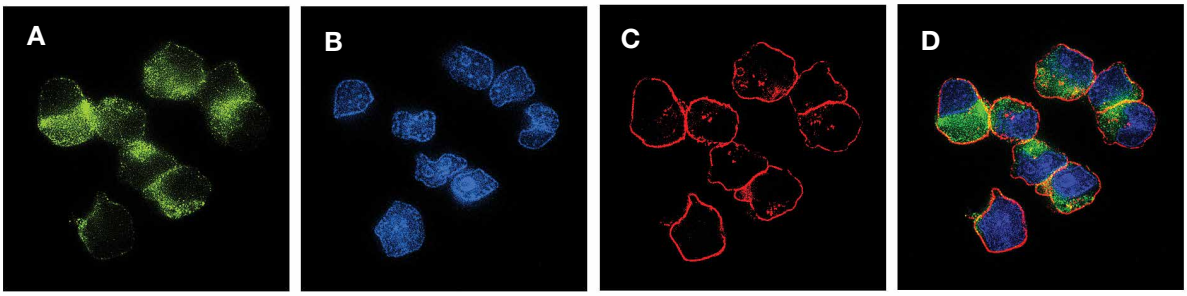



**Figure 6. Expression of DR3 in Ramos cells.** Immunocytochemical fluorescence analysis was performed on fixed and permeabilized Ramos cells for detection of **(A)** endogenous DR3, using Invitrogen™ DR3 Recombinant Rabbit Monoclonal Antibody (Cat. No. 702277, 2 µg/mL) in conjunction with Invitrogen™ Alexa Fluor™ 488 Goat Anti–Rabbit IgG Superclonal™ Recombinant Secondary Antibody (green, Cat. No. A27034, 1:2,000 dilution), **(B)** nuclei, using Invitrogen™ SlowFade™ Gold Antifade Mountant with DAPI (blue, Cat. No. S36938), and **(C)** cytoskeletal F-actin, using Invitrogen™ rhodamine phalloidin (red, Cat. No. R415, 1:300 dilution). **(D)** Composite image.

## Antibodies

- **Invitrogen™ antibodies**—Detect key checkpoint targets with primary, secondary, and custom validated antibodies (Figures 6–8).
  - FOXP3
  - Human TIGIT
  - Human LAG3 (and CD223, PD1, CTLA4, TIM3, and VISTA)
  - ARG1, IDO, and NOS2
  - Granzyme B (and GM11)
- **Invitrogen™ Flow Cytometry Panel Builder**—Incorporate multiple I-O antibodies into one experiment with our Flow Cytometry Panel Builder. This tool is built on a five-step process for multicolor panel design to easily add antibodies and fluorophores with minimal spectral overlap.

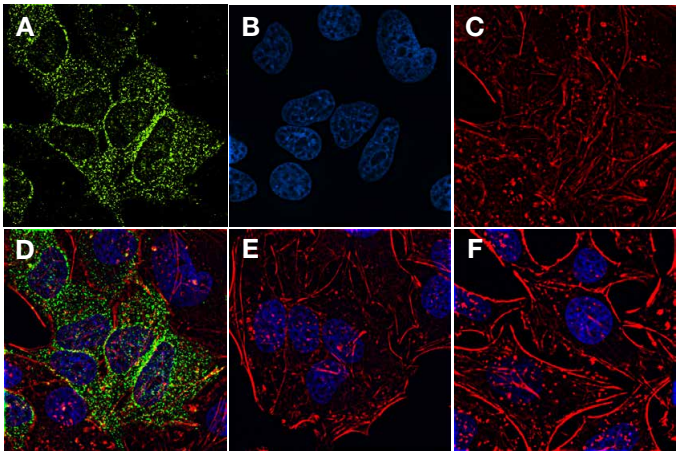



**Figure 7. Expression of IDO in HeLa cells.** HeLa cells were treated with IFN-γ for detection of endogenous IDO using Invitrogen™ IDO Recombinant Polyclonal Antibody (Cat. No. 711778) and Alexa Fluor 488 Goat Anti–Rabbit IgG Superclonal Recombinant Secondary Antibody (Cat. No. A27034, 1:2,000). **(A)** Representative cells stained for IDO1 protein (green). **(B)** Nuclei (blue) stained using SlowFade Gold Antifade Mountant with DAPI (Cat. No. S36938). **(C)** Cytoskeletal F-actin stained using rhodamine phalloidin (Cat. No. R415, 1:300). **(D)** Composite image showing cytoplasmic localization of IDO. **(E)** Untreated cells. **(F)** Control cells.

**Did you know?**

Our antibodies are undergoing a rigorous two-part testing approach recognized with a 2018 CiteAb Award. thermofisher.com/antibodyvalidation

We have a quick, simple search tool for finding the specific antibody you need for your experiments. thermofisher.com/antibodies

If you can't find the antibody you need, we also offer custom antibody services. thermofisher.com/customabs

Design your panel now at thermofisher.com/panelbuilder

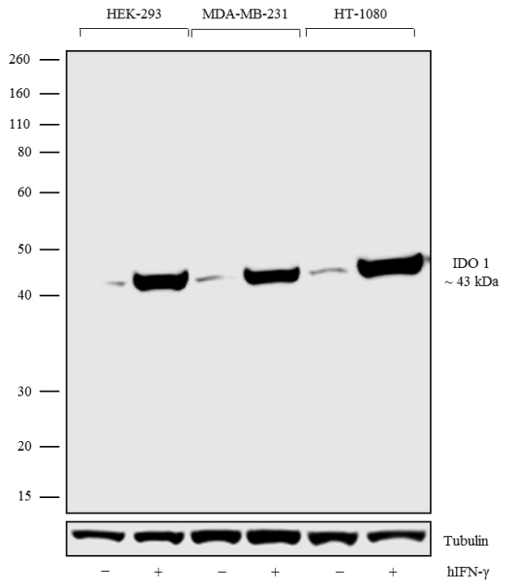



**Figure 8. Western blot of IDO expression.** The indicated cell lines were treated with IFN-γ (50 ng/mL, 24 hr) or left untreated. A 43 kDa band corresponding to IDO that increases with IFN-γ treatment was detected using IDO Recombinant Polyclonal Antibody (Cat. No. 711778, 1 µg/mL) and Invitrogen™ Goat Anti–Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Cat. No. A27036). Cell lysates were electrophoresed using an Invitrogen™ NuPAGE™ 10% Bis-Tris gel (Cat. No. NP0301BOX), Invitrogen™ XCell SureLock™ Mini-Cell (Cat. No. EI0002), and Invitrogen™ Novex™ Sharp Pre-stained Protein Standard (Cat. No. LC5800). Proteins were transferred to a nitrocellulose membrane using the Invitrogen™ iBlot™ 2 Gel Transfer Device (Cat. No. IB21001). Chemiluminescent detection was performed using Thermo Scientific™ Pierce™ ECL Western Blotting Substrate (Cat. No. 32106).

## Quantitative protein analysis

ELISAs, Invitrogen™ ProQuantum™ high-sensitivity immunoassays, Luminex® multiplex platforms, and next-generation immunoassays are routinely used for quantitative assessment of soluble proteins such as cytokines, chemokines, growth factors, and other immunological markers.

- **Invitrogen™ ProcartaPlex™ Immuno-Oncology Checkpoint Markers Panel**—Multiplexed checkpoint analysis using Luminex® xMAP® (multi-analyte profiling) technology enables simultaneous detection and quantitation of multiple secreted proteins (e.g., cytokines, chemokines, and growth factors).
- **Invitrogen™ QuantiGene™ Plex Assay**—Utilizes Luminex xMAP bead technology for multiplexing using a Luminex instrument. Accurately measure RNA from archival tumor sections and achieve quantitative measurement of biomarkers. Choose from our inventory of over 17,000 validated genes to create pathway- and disease-themed panels for biomarker verification. Conserve your sample with our preparation kits for minimal sample while examining many genes in a one-well format.

## Other assay tools

- **Invitrogen™ CellTrace™ assays**—Conduct proliferation measurements based on DNA synthesis or on cellular metabolism parameters. Assays can report cell health, genotoxicity, and inhibition of tumor cell growth during drug development. Find out more at thermofisher.com/celltrace
- **Invitrogen™ Click-iT™ EdU assays**—Provide a simplified, more robust assay for analyzing DNA replication in proliferating cells compared to traditional BrdU methods. Find out more at thermofisher.com/clickitflow
- **Invitrogen™ PrimeFlow™ RNA assays**—Designed for RNA and protein expression analysis as cells change over time or in response to stimuli; assay for any protein when no antibody is available.

## Straight from the scientist

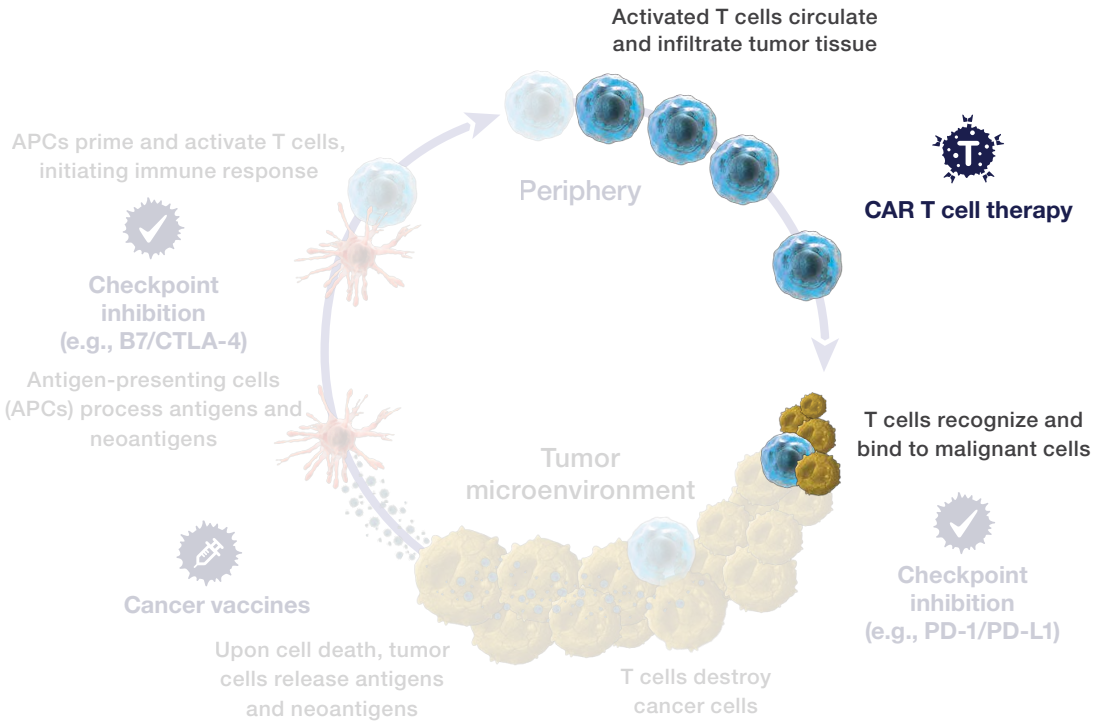
On uncovering checkpoint biomarkers with ProcartaPlex immunoassays for multiplex protein quantitation using the Luminex instrument platform:

"Multiplex measurement of soluble forms of the immune checkpoint receptors and ligands is novel.… Detection of 65 cytokines, chemokines, and growth factors in a single Luminex assay was also a favorable trait as it combines many analytes into a single assay and with small volumes. This diversifies the use of immunoassays based on the Luminex platform for broad biomarker discovery and validation rather than only for testing a specific hypothesis."

Lisa Butterfield, PhD
Professor of Medicine, Surgery, and Immunology;
Director, UPCI Immunologic Monitoring and Cellular Products Laboratory, University of Pittsburgh

# Solutions for CAR T cell therapy

Analyzing the immune repertoire to capture the diversity of TCR rearrangements can help you make significant progress in I-O research. Explore workflow solutions from engineering to sequencing through cell media and reagents.




## Sequencing

- **Next-generation sequencing (NGS) using the Ion GeneStudio™ S5 System series**—Ion Torrent™ semiconductor sequencing enables a broad range of targeted NGS applications with outstanding speed and scalability. With the Ion GeneStudio S5 Systems, researchers can select from five different chips to enable a sequencing throughput of 2–130 million reads per run.
- **Ion Torrent™ Oncomine™ TCR Beta-LR Assay**—Discover a rapid, long-read NGS assay enabling optimization of the function and manufacturing of potentially therapeutic T cells as well as the investigation of markers for immune-mediated adverse events (IMAEs).
- **Sanger sequencing with the Applied Biosystems™ SeqStudio™ Genetic Analyzer**—Utilize TCR sequencing (beta, gamma, alpha) and analysis to assess T cell repertoire diversity. The technology allows any custom TCR sequencing application for sequences up to 700 bp.

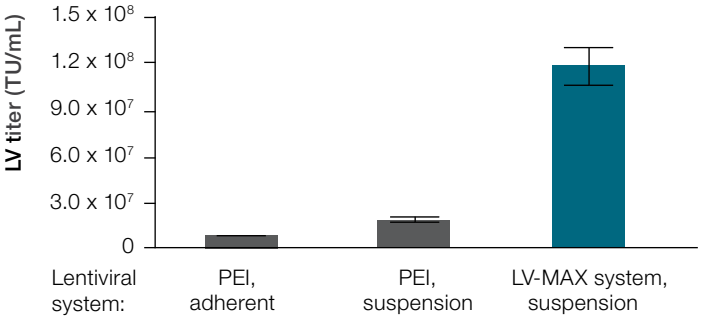
## Engineering

- **Gibco™ LV-MAX™ Lentiviral Production System**—Addresses challenges that exist in adherent and suspension methods for lentiviral vector production by providing a cost-effective and scalable platform to support your current lentiviral vector needs and future large-volume demand. As seen in Figure 9, the LV-MAX system produced 15x more virus than the PEI-mediated system in adherent cells and 10x more virus than in suspension cells, resulting in over 50% cost reduction compared to PEI-based lentiviral production methods.
- **Invitrogen™ TrueGuide™ Synthetic gRNA**—Ready-to-transfect single guide RNA (sgRNA) designed and validated to work with the Invitrogen™ suite of genome editing tools to provide consistent, high-efficiency editing.
- **Invitrogen™ TrueCut™ Cas9 proteins**—Discover our next-generation Cas9 proteins designed to deliver consistently high editing efficiency across a range of gene targets and cell types. Invitrogen™ TrueCut™ Cas9 Protein v2 is well suited for most common research applications, and Invitrogen™ TrueCut™ HiFi Cas9 Protein is designed for applications that are sensitive to off-target effects (Figures 10 and 11).
- **Invitrogen™ Neon™ Transfection System**—Explore a next-generation electroporation device—up to 90% transfection efficiency in primary cells, stem cells, and difficult-to-transfect cells.
- **Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit**—Use this PCR-based method to measure genome editing efficiency.
- **Invitrogen™ GeneArt™ Gene-to-Protein Services**—Discover fast, reliable protein expression and production from mammalian or insect cells. All proteins are produced in-house with a short processing time starting from 30 business days.
- **Gibco™ Human Plasma–Like Medium**—Utilize a physiologically relevant cell culture medium to facilitate growth and expansion of T cells.



**50% cost reduction compared to PEI-based lentiviral production methods.**

**A**




**B**

**LV-MAX Lentiviral Production System cost savings**

| Current production method | Switch to LV-MAX system and save* |
|---|---|
| Adherent PEI | 58% |
| Suspension PEI | 52% |

* Cost comparison based on list price in USD and lentivirus yield of 1 x $10^8$ TU/mL using the LV-MAX system and 1 x $10^7$ TU/mL using PEI-based adherent and suspension methods. Cost consideration includes media, transfection reagents, and culture vessels.

**Figure 9. Increased viral titer compared to other production methods. (A)** Unfiltered lentivirus produced by suspension cells using the LV-MAX Lentiviral Production System was compared with PEI-mediated transfection of lentiviral vectors in adherent HEK 293T/FT cells and suspension HEK 293 cells. The lentiviral titer was determined by transducing HT1080 cells and analyzing GFP-positive cells. **(B)** The LV-MAX system offers higher titer and over 50% cost reduction compared to PEI-based lentiviral production methods.

Find out more at [thermofisher.com/cartcelltherapy](thermofisher.com/cartcelltherapy)

## Achieve up to 90% functional knockout in human primary T cells with TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNA

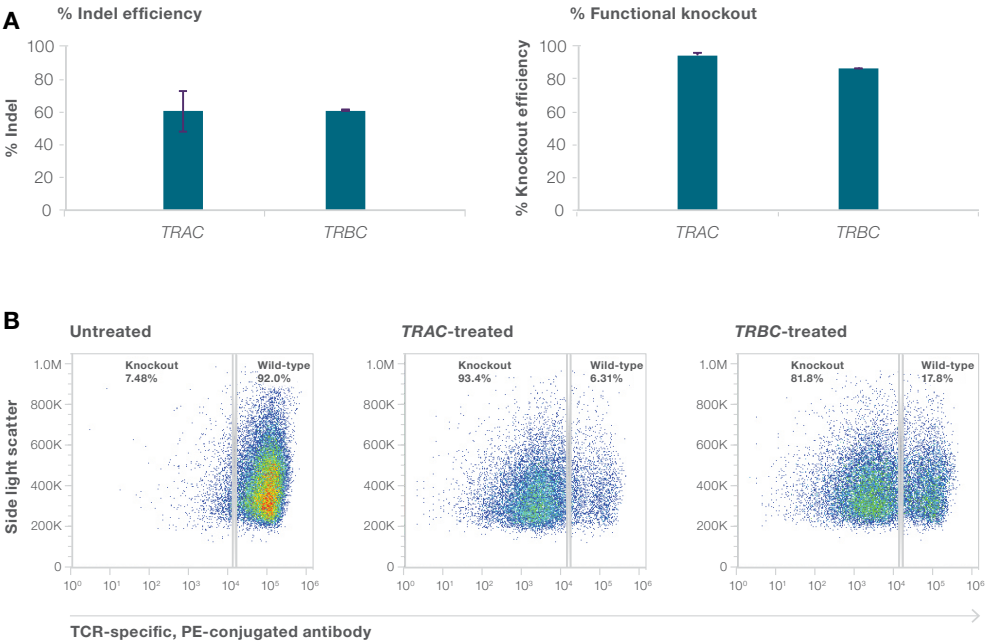



**Figure 10. High-efficiency functional knockout in T cells.** Human T cells were isolated and activated using Invitrogen™ Dynabeads™ magnetic beads and then transfected with TrueCut Cas9 Protein v2 and TrueGuide Synthetic gRNA for T cell receptor alpha (*TRAC*) or beta (*TRBC*) regions using the Neon Transfection System. Following transfection, editing efficiency was measured by **(A)** the GeneArt Cleavage Detection assay, or by **(B)** measuring the percentage of T cell receptor negative ($TCR^-$) cells using the Invitrogen™ Attune™ NxT Flow Cytometer. Cells analyzed by flow cytometry were stained with a TCR-specific antibody conjugated to PE.

## Achieve higher specificity in human primary T cells

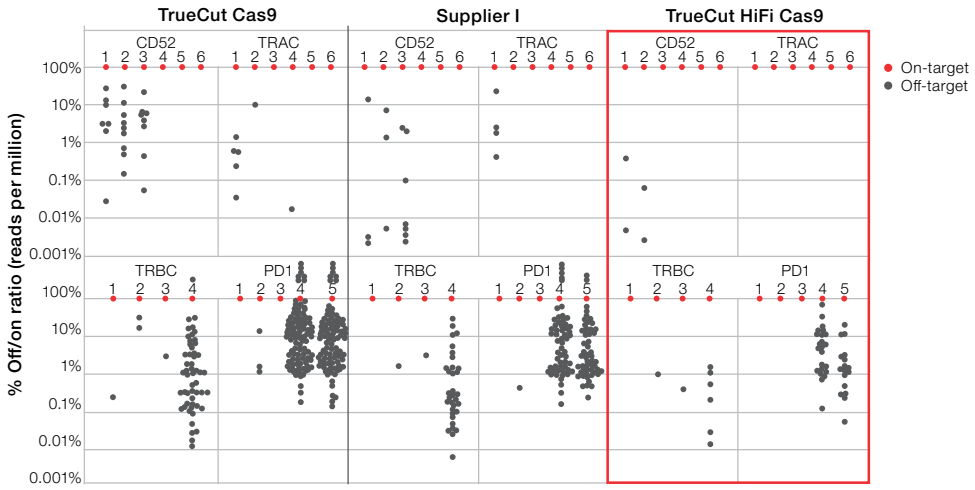



**Figure 11. Fewer off-target events in human primary T cells when using TrueCut HiFi Cas9 Protein.** 21 gRNAs targeting four therapeutically relevant genes (*CD52*, *TRAC*, *TRBC*, and *PD1*) were cotransfected with TrueCut Cas9 Protein v2, TrueCut HiFi Cas9 Protein, or another supplier's high-fidelity Cas9 protein using the Neon Transfection System. Target-enriched GUIDE-Seq (TEG-Seq), an NGS method adapted to Ion Torrent™ sequencing, was used for off-target detection. The red dots are on-target events and normalized to 100%. Gray dots are off-target events plotted against the corresponding off-target to on-target ratio, which represents the risk probability of each off-target event.

Learn more about harnessing CRISPR technology for immuno-oncology research at **thermofisher.com/truecut**

## Translate your cell therapy to the clinic with CTS products

Advancing your cell therapy product from research to clinical applications requires careful selection of materials and thoughtful process development. Gibco™ Cell Therapy Systems (CTS™) products, an extensive selection of cGMP-manufactured media, reagents, and instruments that are designed for cell therapy applications, span the immunotherapy workflow and are designed to facilitate a seamless transition from research to commercialization, with a goal to reduce the time from your initial discovery to an approved cell-based immunotherapy.

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

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

### Isolation and activation

- **Gibco™ CTS™ Rotea™ Counterflow Centrifugation System**—A versatile, closed cell processing system for cell therapy that applies the proven counterflow centrifugation method to a broad range of cell processing applications such as cell isolation and separation, cell washing and concentration, buffer exchange, and more. The instrument's compact footprint, superb performance, process flexibility, and sterile single-use kit allow the system to seamlessly scale your process from research through commercial manufacturing.
- **Gibco™ CTS™ Dynabeads™ CD3/CD28**—Leverage magnetic beads, a trusted technology platform for *ex vivo* T cell isolation, activation, and expansion for immunotherapy [13-15].

### Engineering

- **Gibco™ CTS™ LV-MAX™ Lentiviral Production System**—Addresses challenges that exist across adherent and suspension methods for lentiviral vector production by providing a cost-effective and scalable platform to support your current lentiviral vector needs and future large-volume demand. To streamline your transition from research to clinical scale, we now offer a complete suspension lentiviral vector production system that produces high titers (>1 x $10^8$ TU/mL unconcentrated particles). A smooth ramp-up from research-grade lentiviral vector production to clinical production is essential. To accelerate your development timelines and ease the transition, we offer complementary research-grade and GMP products for one complete solution from research through clinical and commercial production.
- **Gibco™ CTS™ Xenon™ Electroporation System**—A closed, scalable large-scale platform that offers full control over electroporation parameters for optimal performance. The CTS Xenon Electroporation System enables reliable delivery of DNA, RNA, proteins, and other molecules into cells with exceptional cell viability and recovery, leading to efficient transfection and genome editing even in hard-to-transfect cells (Figure 12). Designed to enable electroporation of up to 2.5 x $10^9$ T cells in 25 mL per run for cell therapy process development and manufacturing.
- **Gibco™ CTS™ TrueCut™ Cas9 Protein**—GMP-manufactured Cas9 protein designed to provide consistently high editing efficiency in all tested cell lines, including >90% editing efficiency in primary T cells, to support your therapeutic applications.

## Expansion

- **Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM)**—Use a complete xeno-free formulation proven for clinical success and specifically developed for the growth and expansion of human T lymphocytes.
- **Gibco™ CTS™ AIM V™ Medium**—Try a fully defined, serum-free formulation for proliferation or manipulation of T cells, dendritic cells, and other primary or immortalized cells. Contains L-glutamine and is formulated with or without phenol red and antibiotics, making it ideal for research or manufacturing.
- **Gibco™ CTS™ GlutaMAX™ Supplement**—Get improved growth efficiency and performance of mammalian cell cultures with this animal origin–free (AOF) dipeptide alternative to L-glutamine that has increased stability for improved cell health.
- **Gibco™ CTS™ Immune Cell Serum Replacement**—Get a defined xeno-free formulation proven for clinical use and designed to support expansion of *in vitro*–cultured human T cells when added as a supplement to a basal cell culture medium such as CTS OpTmizer T Cell Expansion SFM or CTS AIM V Medium.

Many of our Gibco™ CTS™ reagents are now offered in a BioProcess Container format suitable for closed-system workflows, so you can put an end to the expensive, risky, and time-consuming manual transfer of media and reagents from bottles to bags.

## Wash and cryopreservation

- **Gibco™ CTS™ Rotea™ Counterflow Centrifugation System**—Streamline and expedite your CAR T cell therapy development with this versatile, closed cell processing system that applies the proven counterflow centrifugation method to a broad range of cell processing applications such as cell washing and concentration, cell isolation and separation, buffer exchange, and more. Easily scale from research through commercial manufacturing with the same instrument.
- **Gibco™ CTS™ DPBS, without calcium chloride, without magnesium chloride**—Maintain the structural and physiological integrity of cells in vitro using this chemically defined and animal origin–free (AOF) medium, suitable for use in clinical research studies.

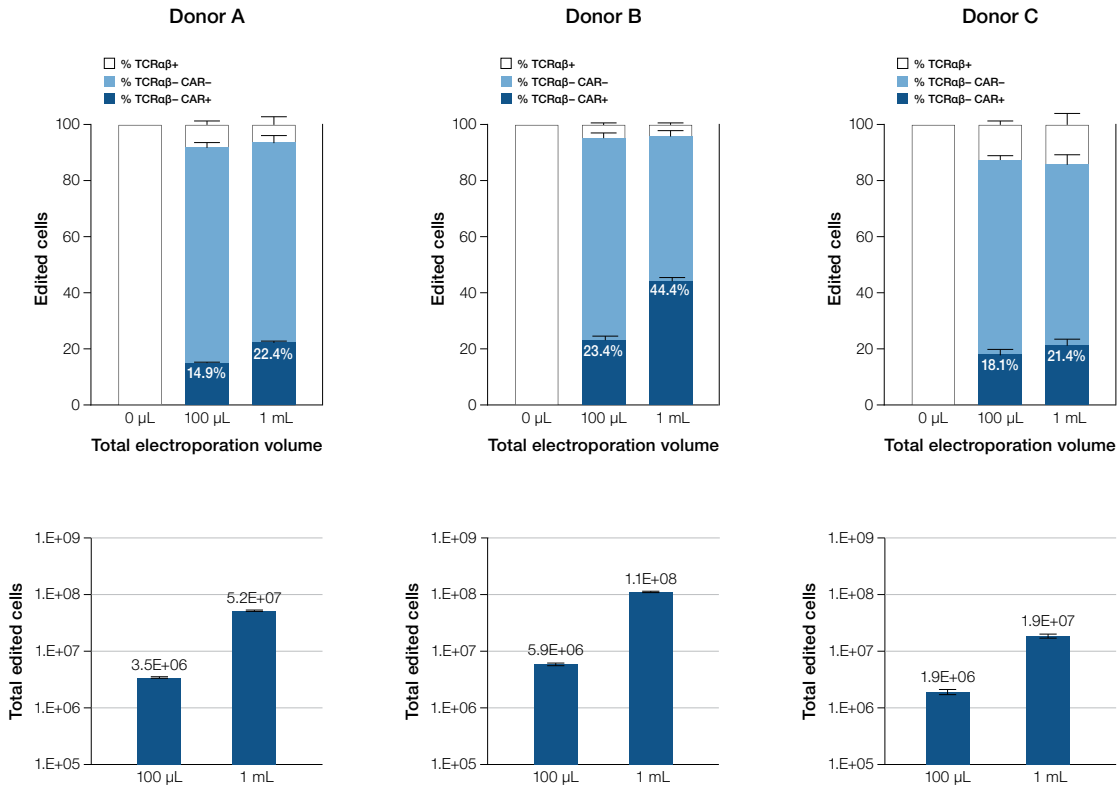



**Figure 12. Gene editing efficiency.** Activated T cells were genetically modified using either the Neon (100 µL) or CTS Xenon (1 mL) transfection system. The cells were modified with ribonucleoprotein (RNP) complexes comprising CTS TrueCut Cas9 Protein and TrueGuide sgRNA targeting the *TRAC* site. The RNP complexes were used in conjunction with a dsDNA CAR construct. Higher knock-in efficiency was observed with the CTS Xenon system than with the Neon system. Data were collected 3 days post-electroporation.

# Gene-modified T cells

Generate CAR T cells with solutions that span the workflow.



## Isolation and activation

**Cell processing**

- CTS Rotea Counterflow Centrifugation System

**Gibco isolation and activation**

- CTS Dynabeads CD3/CD28
- Dynabeads Human T-Expander CD3/CD28
- CTS Dynabeads Treg Xpander

**Gibco instrumentation**

- CTS DynaMag Magnet
- CTS DynaCellect Magnetic Separation System (coming soon)

## Engineering

**Nonviral platforms**

- CTS Xenon Electroporation System
- Neon Transfection System

**Gene editing**

- CTS TrueCut Cas9 Protein
- CRISPR-Cas9 and designer TALEN products and services
- TrueGuide Synthetic gRNA

**Viral platforms**

- CTS LV-MAX Production System

**Confirmation of gene edits**

- BigDye Terminator and BigDye Direct Sanger sequencing reagents
- ExoSAP-IT and BigDye XTerminator reagents
- 3500, SeqStudio, and SeqStudio Flex Genetic Analyzers
- SeqScreener Gene Editing Confirmation App

## Expansion

**Gibco catalog and custom media**

- CTS OpTmizer T Cell Expansion SFM
- CTS OpTmizer T Cell Expansion SFM, no phenol red
- CTS OpTmizer Pro SFM
- CTS AIM V SFM
- CTS AIM V Medium, without phenol red, without antibiotics
- Custom media and services

**Gibco cell culture supplements**

- CTS GlutaMAX-I Supplement
- CTS Immune Cell SR
- Premium FBS

**Thermo Scientific equipment**

- HyPerforma Rocker Bioreactors with bioprocess controllers and software
- Single-use technologies: BioProcess Containers, transfer assemblies, rocker bags
- Herasafe 2030i Biological Safety Cabinets CTS Seriess
- Heracell Vios CR $CO_2$ Incubators CTS Series
- Sorvall X4 and X4F Pro Centrifuges CTS Series

**Gibco growth factors**

- IL-2, IL-4, IL-7, IL-15, GM-CSF

## Wash and cryopreservation

**Gibco wash and concentrate**

- CTS DPBS, without calcium chloride, without magnesium chloride
- CTS Rotea Counterflow Centrifugation System

**Gibco cryopreservation**

- CTS Synth-a-Freeze Medium

**Thermo Scientific cryopreservation**

- CryoMed Controlled-Rate Freezer
- CryoPlus $LN_2$ Storage Systems
- CryoExtra High-Efficiency Cryogenic Storage Systems
- Nunc cryogenic tubes

## Lot release and characterization

**Applied Biosystems assays**

- PureQuant CD8+ T Cell Assay
- PureQuant Treg Assay
- PureQuant Th17 Assay
- MycoSEQ Mycoplasma Detection Kit
- resDNASEQ Human Residual DNA Quantitation Kit
- resDNASEQ Quantitative HEK293 DNA Kit with PrepSEQ Residual DNA Sample Preparation Kit
- AmpFLSTR Identifiler Direct PCR Amplification Kit

**Cell line authentication**

- IdentiFiler CLA STR analysis kit
- GlobalFiler CLA STR analysis kit

**Invitrogen instrumentation**

- Attune Cytometric Software, compliant with 21 CFR Part 11
- CytKick Autosampler

**Invitrogen assays**

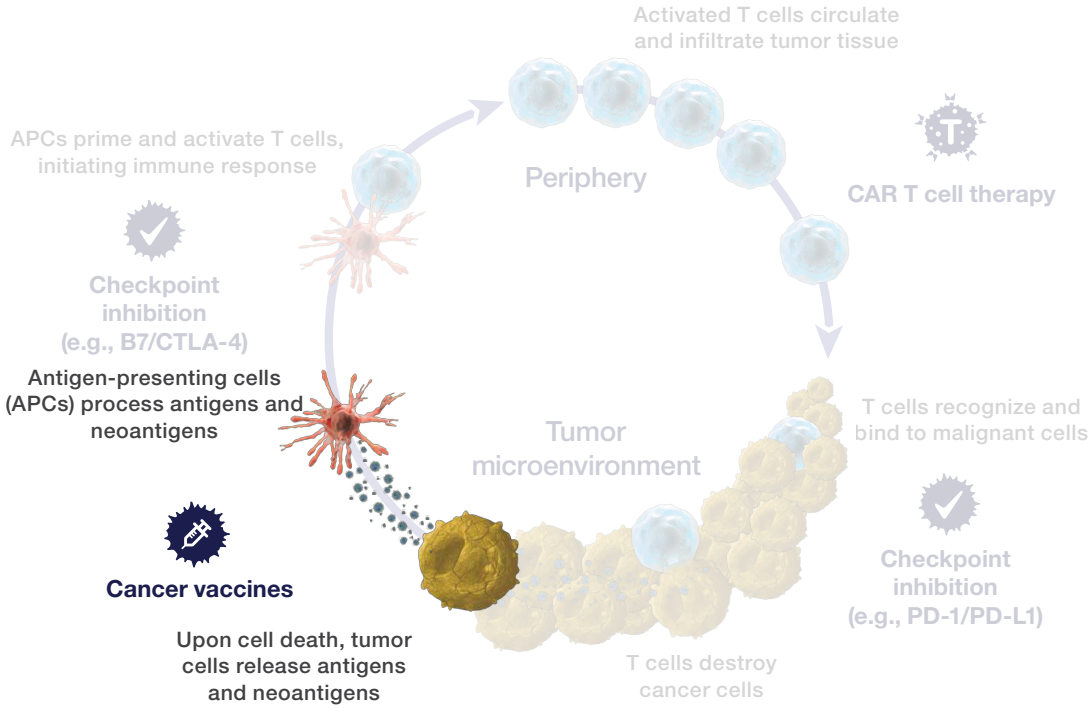
- eBioscience Essential Human Treg Phenotyping Kit
- eBioscience Essential Human Th1/Th17 Phenotyping Kit
- eBioscience Essential Human T Cell Phenotyping Kit
- CyQUANT LDH Cytotoxicity Assay

**Thermo Scientific assay**

- Pierce Chromogenic Endotoxin Quant Kit

# Solutions for cancer vaccine research

Cancer vaccine research is rapidly evolving due to the potential behind combination therapies and personalized approaches. This shows promise beyond conventional cancer treatments such as radiation and surgery. As research advances, our solutions for this promising field will be there to facilitate the journey onward.




## Genomic biomarker discovery and verification tools

- **Applied Biosystems™ OncoScan™ CNV Assay**—Explore a microarray-based whole-genome copy number assay for solid tumor samples that enables the detection of a wide variety of somatic copy number aberrations. Accurately assess gains, losses, loss of heterozygosity (LOH) and copy-neutral LOH, chromothripsis, aneuploidy, and more to identify important copy number–based biomarkers.
- **Applied Biosystems™ CytoScan™ HD Suite**—Utilize a microarray-based whole-genome copy number assay for hematological malignancy samples that enables the detection of a wide variety somatic copy number aberrations. Accurately assess gains, losses, LOH and copy-neutral LOH, chromothripsis, aneuploidy, and more to identify important copy number–based biomarkers.

## Gene synthesis services

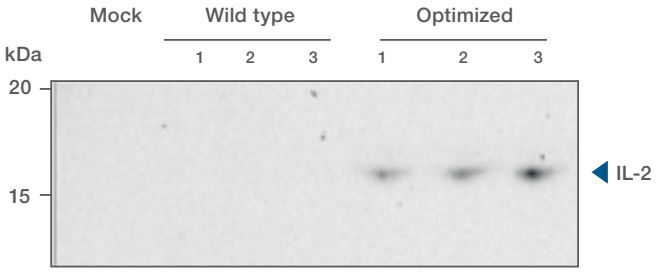
- **Invitrogen™ GeneArt™ Gene Synthesis**—Leverage outstanding sequence optimization and gene synthesis services, optimized protein expression services, or outsource the entire process of protein and cell line production with GeneArt services. Maximize protein yield with Invitrogen™ GeneOptimizer™ software that generates sequence variants with enhanced mRNA stability and translational efficiency. Figure 13 shows an example of increased expression by optimized gene sequences in different host cells [16].

Find out more at [thermofisher.com/vaccineresearch](thermofisher.com/vaccineresearch)

## Engineering

- **Invitrogen™ PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit**—Achieve isolation of endotoxin-free plasmid DNA using an enhanced anion exchange membrane, in as little as 90 minutes.
- **Invitrogen™ Lipofectamine™ 3000 Transfection Reagent**—Get high transfection efficiency with a broad spectrum of challenging cell types.
- **Invitrogen™ MEGAscript™ T7 Transcription Kit**—Get >10 times higher RNA yield than from conventional *in vitro* transcription reactions with this ultrahigh-yield *in vitro* transcription kit.
- **Invitrogen™ TransfectionSelect™ product selection tool**—Answer a few simple questions about your transfection, and the tool quickly and accurately generates recommended solutions, protocols, and citations to meet your research needs. Go to thermofisher.com/transfectionselect.





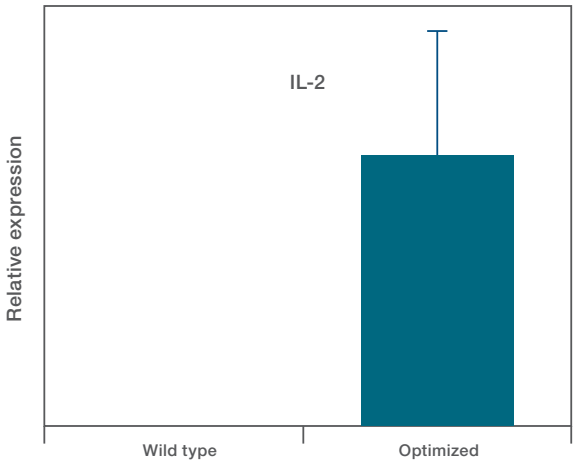




**Figure 13. The combination of GeneArt expression optimization and advanced Gibco expression systems (e.g., Expi293F cells) usually leads to higher overall project reliability and expression yields than can be obtained with nonoptimized genes.** This is achieved via the GeneArt GeneOptimizer algorithm for protein expression optimization, which determines the optimal gene sequence for your expression experiments. Common pain points associated with protein expression, such as yield, are addressed in a rational and systematic way using a multiparameter approach. Optimization has been experimentally proven to increase protein expression rates up to 100-fold in a variety of host systems [16].

## White paper

**Looking to optimize your protein expression for antibody production?**

Get a 4-fold average increase for monoclonal antibodies and other proteins.

Find out more at thermofisher.com/genetoprotein

# Characterize and verify

Enhance the analysis of genes, proteins, and cells, from confirming targets and expression to understanding the impact of mechanisms of action.

## Targeted genetic analysis

Confirm and quantify genetic changes for a wide range of selected DNA targets that are key to your research.

### Isolate high-quality genomic DNA or viral DNA from a range of sample types for use in all common molecular biology applications

- Invitrogen™ DNAzol™ Reagent for genomic DNA isolation
- Invitrogen™ PureLink™ genomic DNA purification kits
- Thermo Scientific™ KingFisher™ purification systems for DNA and RNA with Applied Biosystems™ MagMAX™ nucleic acid isolation kits
- Applied Biosystems™ Arcturus™ LCM Instrument
- Invitrogen™ Qubit™ fluorometers and Qubit™ DNA assay kits
- Thermo Scientific™ NanoDrop™ One/One$^{C}$ Microvolume UV-Vis Spectrophotometer

**Endpoint PCR**
Amplify DNA sequences for subsequent molecular biology applications

**Amplify target DNA**

- Applied Biosystems™ SimpliAmp™, VeritiPro™, and ProFlex™ thermal cyclers
- Applied Biosystems™ MicroAmp™ EnduraPlate™ 96- and 384-well plates with barcode
- Invitrogen™ custom DNA oligos
- Invitrogen™ Platinum™ II *Taq* DNA Polymerase and Platinum™ SuperFi™ II DNA Polymerase

**Analyze data by electrophoresis**

- Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System
- E-Gel™ CloneWell™ II, SizeSelect™ II, and EX Agarose Gels
- Invitrogen™ TrackIt™ ladders
- Invitrogen™ UltraPure™ Agarose
- Invitrogen™ SYBR™ Safe dyes

**Real-time PCR**
Accurately analyze specific DNA targets

**Select assays**

- Applied Biosystems™ TaqMan™ SNP Genotyping Assays
- Applied Biosystems™ TaqMan™ Copy Number Assays
- Applied Biosystems™ TaqMan™ Mutation Detection Assays
- Applied Biosystems™ TaqMan™ Flexible Content Panels
- Applied Biosystems™ TaqMan™ liquid biopsy dPCR assays

**Prepare reaction**

- Applied Biosystems™ TaqPath™ ProAmp™ Master Mix
- Applied Biosystems™ QuantStudio™ 3D Digital PCR Master Mix v2

**Run real-time or digital PCR**

- Applied Biosystems™ QuantStudio™ 3, 5, 6 Flex, 7 Flex, and 12K Flex systems
- Applied Biosystems™ QuantStudio™ 3D Digital PCR System

**Analyze data**

- qPCR Analysis Modules
- Applied Biosystems™ TaqMan™ Genotyper Software
- Applied Biosystems™ CopyCaller™ Software
- AlleleTyper™ Software

**Sanger sequencing**
Verify DNA sequence rapidly using proven DNA sequencing technology

**PCR amplification**

- Applied Biosystems™ AmpliTaq™ DNA Polymerase
- Invitrogen™ Primer Designer™ Tool for PCR and Sanger sequencing

**PCR cleanup**

- Applied Biosystems™ ExoSAP-IT™ *Express* reagent

**Cycle sequencing**

- Applied Biosystems™ BigDye™ Terminator cycle and direct cycle sequencing kits

**Sequencing cleanup**

- Applied Biosystems™ BigDye™ XTerminator™ Purification Kit

**Sanger sequencing instrumentation**

- Applied Biosystems™ SeqStudio™ Genetic Analyzer
- Applied Biosystems™ 3500 Genetic Analyzer

**Analyze data**

- Applied Biosystems™ Sanger Analysis Modules
- Applied Biosystems™ Minor Variant Finder Software

**NGS**
Sequence hundreds to thousands of targets at high depth

**Select targets**

- Ion AmpliSeq™ Panels
- Ion AmpliSeq™ Designer tool for custom designs
- Ion Torrent™ Oncomine™ assays for immuno-oncology and liquid biopsy research

**Construct library and/or prepare template**

- Ion Chef™ System
- Ion AmpliSeq™ Kit for Chef DL8

**Sequence**

- Ion GeneStudio™ S5 systems

**Analyze data**

- Torrent Suite™ Software
- Ion Reporter™ Software
- Ion Torrent™ Oncomine™ Knowledgebase Reporter

# Targeted gene expression analysis

Confirm and quantify RNA targets at the gene, exon, or noncoding RNA level.

## Isolate the highest-quality cellular RNA, viral RNA, or miRNA from a range of sample types for direct use in all common molecular biology applications

- Invitrogen™ RNA*later*™ Stabilization Solution
- Invitrogen™ RNase*Zap*™ RNA Decontamination Solution
- Invitrogen™ TRIzol™ Reagent for DNA, RNA, or protein isolation
- Invitrogen™ PureLink™ RNA purification kits
- Invitrogen™ *mir*Vana™ miRNA isolation kits
- Applied Biosystems™ Arcturus™ LCM Instrument
- KingFisher purification systems for DNA and RNA with MagMAX nucleic acid purification kits
- Qubit fluorometers, Qubit RNA and miRNA assay kits
- NanoDrop One/One$^{C}$ spectrophotometers

### RT-PCR

Confirm and compare gene expression for small sample numbers by RT-PCR

**Synthesize and amplify cDNA**

- Invitrogen custom DNA oligos
- Invitrogen™ SuperScript™ IV One-Step RT-PCR System
- SuperScript IV One-Step RT-PCR System with ezDNase™ Enzyme
- MicroAmp EnduraPlate 96- and 384-well plates with barcode
- SimpliAmp, VeritiPro, and ProFlex thermal cyclers

**Analyze data by electrophoresis**

- E-Gel Power Snap Electrophoresis System
- E-Gel CloneWell II, SizeSelect II, and EX Agarose Gels
- TrackIt ladders
- UltraPure Agarose
- SYBR Safe dyes
- SYBR Gold dyes

### Real-time RT-PCR

Quantify gene expression with speed and accuracy

**Select assays**

- Applied Biosystems™ TaqMan™ Gene Expression Assays
- Applied Biosystems™ TaqMan™ Advanced miRNA Assays
- TaqMan Flexible Content Panels

**Perform reverse transcription**

- Applied Biosystems™ TaqMan™ Fast Advanced Master Mix
- Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix

**Run real-time or digital PCR**

- QuantStudio 3, 5, 6 Flex, 7 Flex, and 12K Flex systems
- QuantStudio 3D Digital PCR System

**Analyze data**

- qPCR Analysis Modules
- Applied Biosystems™ ExpressionSuite™ Software

### NGS

Confirm thousands of biomarkers quickly and reproducibly

**Select targets**

- Ion AmpliSeq Panels
- Ion AmpliSeq Designer tool for custom designs
- Oncomine assays for immuno-oncology and liquid biopsy research

**Construct library and/or prepare template**

- Ion Chef System
- Ion AmpliSeq Kit for Chef DL8

**Sequence**

- Ion GeneStudio S5 systems

**Analyze data**

- Torrent Suite Software
- Ion Reporter Software
- Oncomine Knowledgebase Reporter



Order target-specific primers at [thermofisher.com/oligos](thermofisher.com/oligos)

## Solution spotlight: genetic analysis tools

**Ion Torrent™ Oncomine™ Immune Response Research Assay**—This panel was carefully selected to monitor the tumor microenvironment (TME). The assay can be used for identification of biomarkers and studying mechanisms of action and other interactions emanating from combination therapy experiments. In a head-to-head comparison with assays from other suppliers, the assay detected lower expressors associated with T cell receptor (TCR) signaling and checkpoint inhibitors, thereby allowing researchers to focus on the correct TCR and the most suitable immunotherapy.

**Ion Torrent™ Oncomine™ Tumor Mutation Load (TML) Assay**—The Oncomine TML Assay covers 1.7 Mb across 409 cancer-driven genes relevant across major cancer types, and requires as little as 20 ng of tumor DNA with a 3-day workflow with streamlined analysis. The assay highly correlates with exome mutation counts and thereby obviates the need for whole-exome sequencing, allowing a higher percentage of samples to be evaluated while conserving precious samples for additional biomarker assessment.

**Ion Torrent™ Oncomine™ TCR Beta-LR Assay**—Utilizing a newly developed long-read sequencing technology, the Oncomine TCR Beta-LR Assay is designed to efficiently capture all three complementarity-determining regions of the TCR beta chain (CDR1, CDR2, CDR3) with high accuracy. This assay enables key applications such as predictive or prognostic biomarker discovery, T cell characterization, and identification of variable gene polymorphisms from samples such as RNA extracted from whole blood, fresh-frozen tissue, or sorted cells. The identification of rare and abundant clones can be achieved with as little as 10 ng RNA input. The use of RNA template allows sequencing of productive and relevant variable (V), diversity (D), and joining (J) rearrangements—improving the identification of rare clones.

**Ion Torrent™ Oncomine™ TCR Beta-SR Assay**—The latest addition to the Ion Torrent immuno-oncology NGS portfolio, the Oncomine TCR Beta-SR Assay specifically interrogates the CDR3 region of the TCR beta chain. Compatible with both FFPE DNA and RNA, this short-read sequencing assay enables characterization of the immune status, and detection of T cell minimal residual disease (MRD) in the peripheral blood. Requiring low sample input, this assay offers a 2-day turnaround time complete with user-friendly informatics for accurate clonality and CDR3 TCR beta chain sequence assessment without interference from primer bias.

thermofisher.com/ngs-immuneresponse

**Ion GeneStudio S5 systems**—Get from DNA to data with less than 45 minutes of hands-on time. With simple cartridge-loaded reagents and a straightforward user interface, the Ion GeneStudio S5 systems make NGS fast and easy—ideal for any cancer or inherited disease research lab. Ion Torrent™ technology has been referenced in over 4,000 publications to date. Now you can drive your research forward using this highly cited technology with the latest innovations in benchtop NGS.

thermofisher.com/genestudio

**Applied Biosystems™ TaqMan™ Gene Expression Assays**—Over 1.8 million predesigned TaqMan Gene Expression Assays covering more than 30 species are available in single-tube, 96-well plate, 384-well microfluidic card, and Applied Biosystems™ OpenArray™ formats. These assays are referenced in tens of thousands of publications and are considered the gold standard for gene expression quantification.

thermofisher.com/taqmangex

# Cell analysis

Understand the impact of external and internal perturbations on cellular phenotypes and behavior.

| **Observe**<br>Compact, all-in-one, transmitted-light systems for easy cell, colony, and tissue identification and visualization, and stem cell colony picking | **Count**<br>Accurate and automated counting, monitoring of fluorescent protein expression in cells, and viability studies of cells in just a few seconds | **Visualize**<br>Multicolor fluorescence detection in cells and tissues, plate scanning, z-stacks, and time-lapse studies | **Analyze: Flow cytometry**<br>Run multicolor samples faster and achieve greater resolution with Invitrogen™ acoustics-assisted focusing technology—with little fear of sample loss due to clogging | **Analyze: Quantitative imaging**<br>Integrate high-content screening and analysis into your workflow and analyze up to 500,000 phenotypic measurements in less than 5 minutes |
|---|---|---|---|---|
| • Invitrogen™ EVOS™ XL Core, FLoid™, M5000, and M7000 Imaging Systems<br>• Invitrogen™ Click-iT™ colorimetric IHC detection kits<br>• Invitrogen™ primary and secondary antibodies for IHC<br>• Thermo Scientific™ Nunc™ cell culture plastics<br>• Gibco™ cell culture media | • Invitrogen™ Countess™ 3 Automated Cell Counter<br>• Invitrogen™ Countess™ 3 FL Automated Cell Counter<br>• Countess Reusable Slide<br>• Countess Cell Counting Chamber Slides<br>• Invitrogen™ EVOS™ LED light cubes<br>• Invitrogen™ ReadyProbes™ reagents<br>• Invitrogen™ LIVE/DEAD™ reagents | • Invitrogen™ EVOS™ FL Imaging System<br>• Invitrogen™ EVOS™ FL Auto 2 Imaging System with optional Celleste™ Image Analysis Software<br>• Invitrogen™ EVOS™ Onstage Incubator<br>• Invitrogen primary and secondary antibodies for IHC<br>• Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus antibodies<br>• Invitrogen™ fluorescent reagents<br>• Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader<br>• Thermo Scientific™ Nunc™ microplates for cell-based assays<br>• Invitrogen™ Alexa Fluor™ Antibody Labeling Kits | • Invitrogen™ Attune™ CytPix™ Flow Cytometer<br>• Invitrogen™ Attune™ NxT Flow Cytometer<br>• Invitrogen™ Click-iT™ EdU assays<br>• Invitrogen™ CellTrace™ cell proliferation kits<br>• Invitrogen™ Vybrant™ DyeCycle™ stains<br>• Invitrogen™ FIX & PERM™ Cell Permeabilization Kit<br>• Invitrogen™ Alexa Fluor™, Qdot™, and eBioscience™ Super Bright antibodies<br>• Invitrogen™ PrimeFlow™ RNA assays | • Thermo Scientific™ CellInsight™ CX5, CX7, and CX7 LZR Pro HCS/HCA systems<br>• Invitrogen™ HCS CellMask™, NuclearMask™, and CellTrace™ stains<br>• Invitrogen™ Alexa Fluor™ Plus secondary antibodies<br>• Invitrogen™ ViewRNA™ Cell Plus Assay |



## Discover our cell imaging and analysis capabilities

- Custom services available to create uniquely labeled cell imaging reagents

Visit the Molecular Probes School of Fluorescence at **thermofisher.com/mpsf**

## Solution spotlight: EVOS M7000 Imaging System


Multicolor cellular imaging gives significant information about the cells and biological systems being studied. In addition to various protein levels, imaging also gives information on spatial relationships and other cellular readouts. One such readout is hypoxia. Cellular responses to reduced oxygen (hypoxic conditions) have been linked to a wide range of human pathologies, including tumor development, atherosclerosis, inflammation, and abnormal angiogenesis. Although the importance of hypoxia in inducing these conditions is well known, creating model systems to accurately control the hypoxic conditions is extremely difficult for most researchers. Until recently, to do this effectively, access to elaborate imaging systems that allow maintenance and precise control of temperature, humidity, and gases ($CO_2$ and $O_2$) during an experiment was needed. The EVOS M7000 Imaging System with EVOS Onstage Incubator provides a solution to this situation. This environmental chamber allows the precise control of oxygen levels, thereby delivering an effective system for evaluating cellular responses to hypoxia by long-term fluorescent live-cell imaging.

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

# Solution spotlight: high throughput, safety, and spectrally unmixed cell sorting with the Bigfoot Spectral Cell Sorter

## High throughput

With sort rates exceeding 70,000 events per second (eps) and analysis rates of more than 100,000 eps, the Bigfoot Spectral Cell Sorter is lightning fast. This sorter is capable of 6-way sorting into tubes, multi-way sorting into 96-well and 384-well plates, or straight-down sorting into 1,536-well plates. In addition, virtual 18-way sorting allows researchers to separate multiple populations from a single sample or different samples in sets of 6 populations. For bulk sorts, the InfiniSort capability lets you perform multiple tube or plate sorts in sequence.

The Bigfoot Spectral Cell Sorter can sort a 96-well plate in less than 8 seconds, and a 384-well plate in less than 11 seconds. Plate sorting is not only fast but also remarkably accurate. Using the HRP method, with visual confirmation of droplet deposition through the colorimetric conversion of TMB substrate, we have shown that a single droplet can be sorted in small volumes into both 96-well and 384-well PCR plates with 100% targeting accuracy.

## Safety

The Bigfoot Spectral Cell Sorter features an integrated biocontainment enclosure that provides personnel and product protection similar to a Class II biosafety cabinet.

Test procedures and criteria laid out within NSF49 and EN12469 can be utilized to demonstrate performance. The custom-designed enclosure protects operators and samples from aerosols without compromising high-parameter sorter performance or impacting workflow. A separate aerosol management system meets ISAC guidelines for cell sorters.

## Spectral sorting applications

The Bigfoot Spectral Cell Sorter is especially well suited for sorting applications that require numerous parameters or high throughput.

- **Immunology**—Immunophenotyping parameters have increased as researchers study cell subsets beyond major immune subgroups and effector and memory cells.
- **Cell and gene therapy research**—Therapeutic candidates such as stem cells and CAR T cells must be efficiently sorted both pre- and post-manipulation.
- **Gene editing**—Sort cells of interest prior to editing, and collect populations of cells edited by CRISPR technology or other methods.
- **Gene sequencing**—With output flexibility, the Bigfoot Spectral Cell Sorter can sort single cells directly onto a 10x Genomics chip.

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

# Solution spotlight: Attune CytPix Flow Cytometer

## Advance I-O research with high-performance capabilities

- The Invitrogen™ Attune™ CytPix™ Flow Cytometer has a high-speed brightfield camera that records images of individual events coming through the flow cell. The camera and Attune Cytometric Software help ensure that events recorded by the detector are single cells as opposed to doublets, clumps, or debris. This is crucial in cell and gene therapy research applications (Figure 14).
- With the Attune CytPix Flow Cytometer, you can highlight structural features of large populations in record time. Using image gallery view, you can scan your cell populations rapidly for outliers. This allows you to adjust your gates, if you wish, to include cells of interest while excluding aggregates, unwanted cells, and debris.
- Highest level of data fidelity featuring acquisition of 35,000 events/second and 1 mL/min sample flow rate with acoustics-assisted hydrodynamic focusing.
- New applications to explore difficult samples (including digested tumor samples) without worrying about losing your precious samples, with clog-resistant engineering at ultralow coincidence and abort rates.
- Simplified sample prep for an optimized workflow; perform immunophenotyping analysis on minimally processed samples, reducing the time-intensive traditional sample prep protocols from 10 steps to 3 straightforward steps.

## CytKick Autosamplers allowing walkaway automation

- Two models of autosamplers are available to deliver walkaway automation seamlessly integrated with your Attune CytKick Flow Cytometer for increased productivity. Improve workflow efficiency by choosing the autosampler option that best fits your throughput and experiment requirements.


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

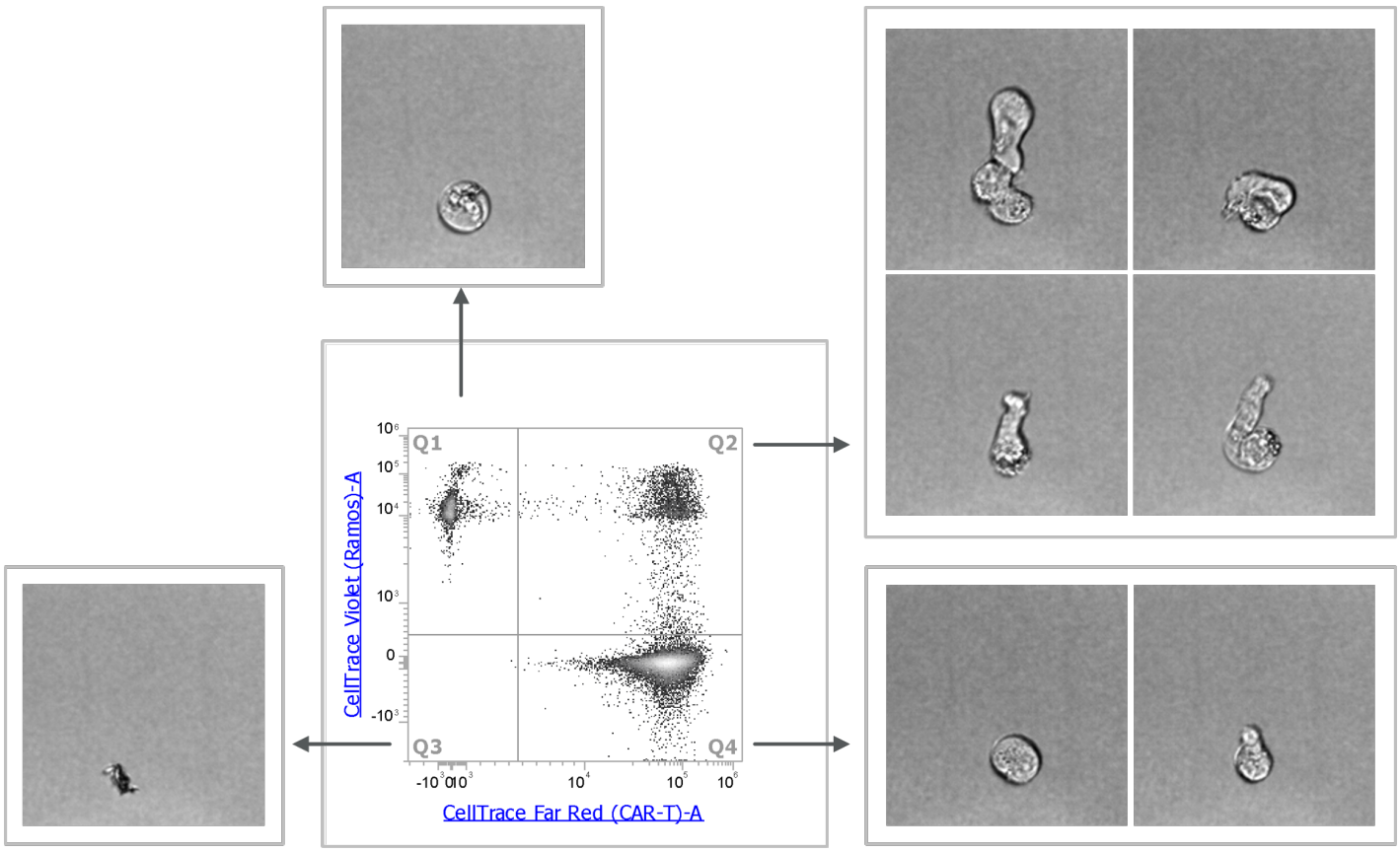



**Figure 14. Photographic evidence for engineered cell potency.** Freshly labeled CAR T cells (labeled with Invitrogen™ CellTrace™ Far Red dye) and Ramos cells (labeled with Invitrogen™ CellTrace™ Violet dye) were incubated at a 1:1 ratio for 1 hr at 37°C. The CAR T cells were engineered to express a single-chain variable fragment targeting CD19 (similar to Juno JCAR019 cells). All samples were unfiltered prior to analysis. Samples were acquired at 200 µL/min, >8 x $10^5$ cells/mL. Q1: Ramos cells without interaction with CAR T cells. Q2: Immune synapse between the two cell phenotypes. Q3: Debris/double-negative events. Q4: CAR T cells without interaction with Ramos cells.

# Solution spotlight: CellInsight CX7 LZR Pro High Content Screening (HCS) Platform

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

In this example, high-content imaging is used to assess cell health and cytotoxicity after "painting" different cellular components (Figure 15).

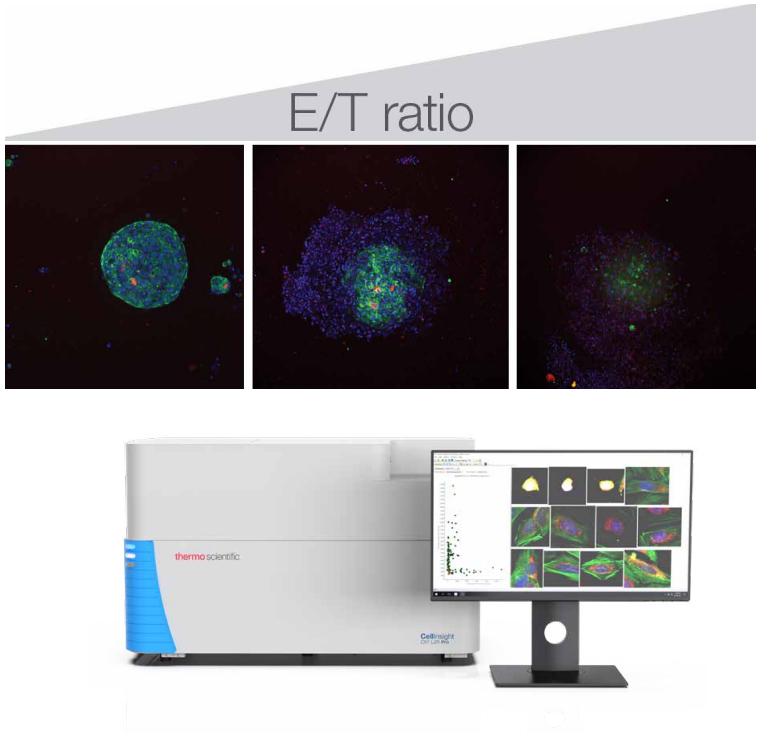



**Figure 15. Cell painting multiparameter phenotyping using the CellInsight CX7 LZR Pro HCS Platform and the Invitrogen™ Image-iT™ Cell Painting Kit.** U2OS cells were labeled with Thermo Scientific™ Hoechst™ 33342 Solution; Invitrogen™ Concanavalin A, Alexa Fluor™ 488 Conjugate; Invitrogen™ SYTO™ 14 Green Fluorescent Nucleic Acid Stain; Invitrogen™ Wheat Germ Agglutinin, Alexa Fluor™ 555 Conjugate; Invitrogen™ Alexa Fluor™ 568 Phalloidin; and Invitrogen™ MitoTracker™ Deep Red FM Dye. After cell staining, images were acquired with the CellInsight CX7 LZR Pro HCS Platform using the 20x 0.8 NA X-line objective lens, and analyzed using the cell painting bioapplication in Thermo Scientific™ HCS Studio™ 5.0 Cell Analysis software.

Find out more at **thermofisher.com/cx7**

# Protein analysis

Analyze the identity, function, and level of expression of key proteins.

| Isolation and cleanup | Separate | Transfer | Detect | Quantify | Modify |
|---|---|---|---|---|---|
| • Chromatography media<br>• Dialysis products<br>• Desalting products<br>• Concentrator devices<br>• Magnetic beads | • Precast protein gels<br>• Pour-your-own protein gel system<br>• Protein ladders<br>• Gel tanks<br>• Power supplies | • Buffers<br>• Membranes<br>• Transfer systems | • Western blotting reagents and devices<br>• Chemiluminescent substrates<br>• Western blot imaging systems | • Total protein and immunoassays<br>• Mass spectrometry reagents for depletion, calibration, and labeling | • Protein crosslinkers<br>• Bioconjugation reagents |
| • Thermo Scientific™ Pierce™ cell lysis solutions<br>• Thermo Scientific™ Pierce™ reagents, LC-MS grade<br>• Thermo Scientific™ Pierce™ protein concentrators<br>• Thermo Scientific™ POROS™ chromatography and purification resins<br>• Thermo Scientific™ Slide-A-Lyzer™ dialysis products<br>• Thermo Scientific™ Zeba™ desalting columns<br>• KingFisher purification systems for DNA, RNA, and protein purification<br>• Invitrogen™ Dynabeads™ magnetic beads for immunoprecipitation | • Invitrogen™ Novex™, Bolt™, and NuPAGE™ precast gels<br>• Invitrogen™ SureCast™ Gel Handcast System<br>• Thermo Scientific™ PageRuler™ and Spectra™ protein ladders<br>• Invitrogen™ Mini Gel Tank<br>• Invitrogen™ SureLock™ Tandem Midi Gel Tank<br>• Invitrogen™ PowerEase™ Touch power supplies | • Invitrogen™ and Thermo Scientific™ Pierce transfer buffers<br>• Invitrogen™ nitrocellulose membranes<br>• Invitrogen™ PVDF membranes<br>• Invitrogen™ iBlot™ 2 Blotting System<br>• Invitrogen™ Power Blotter | • Invitrogen™ Bandmate™ Automated Western Blot Processor<br>• Invitrogen™ iBind™ western devices<br>• Invitrogen™ primary antibodies and HRP secondary antibodies<br>• Alexa Fluor™ and Alexa Fluor™ Plus secondary antibodies<br>• Thermo Scientific™ SuperSignal™ substrates<br>• Invitrogen™ iBright™ Imaging Systems | • Invitrogen™ ProQuantum™ high-sensitivity immunoassay kits<br>• Thermo Scientific™ Pierce™ Rapid Gold BCA and Coomassie protein assays<br>• Invitrogen™ ELISA kits and ProcartaPlex™ multiplex assays<br>• Thermo Scientific™ Varioskan™ LUX and Fluoroskan™ microplate readers<br>• Invitrogen™ Qubit™ fluorometers and protein assay kits<br>• Thermo Scientific™ TMT™ isobaric labeling, proteases, peptide enrichment, calibration solutions, and standards<br>• Thermo Scientific™ SILAC reagents | • Thermo Scientific™ Pierce™ crosslinking, labeling, and bioconjugation reagents<br>• Invitrogen™ SiteClick™ Antibody Azido Modification Kit<br>• Invitrogen™ Alexa Fluor™ dyes |

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- Increase the reproducibility of your western blot experiments
- Get more information from your samples with our cutting-edge mass spectrometry reagents

# Ordering information

### Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|
| Clariom D Pico Assay, human | 30 assays | 902925 |
| Clariom S Pico Assay, human | 30 assays | 902929 |
| CytoScan HD Array Kit and Reagent Kit Bundle | 24 assays | 901835 |
| OncoScan CNV Assay | 24 assays | 902695 |
| TaqMan Array Human Immune Panel | 4 cards | 4370573 |
| TaqMan Array Human Immune Response Plate | 1 plate | 4414073 |
| GeneArt Genomic Cleavage Detection Kit | 20 rxn | A24372 |
| LV-MAX Lentiviral Production System Starter Kit | 1 kit (0.3 L) | A35684 |
| CTS Immune Cell Serum Replacement | 50 mL | A2596101 |
| CTS OpTmizer T Cell Expansion Serum-Free Medium (SFM) | 1 L | A1048501 |
| CTS Dynabeads CD3/CD28 | 10 mL | 40203D |
| Expi293 Expression System Kit | 1 kit | A14635 |
| ExpiCHO Expression System Kit | 1 kit | A29133 |
| Lipofectamine 3000 Transfection Reagent | 0.1 mL | L3000001 |
| | 5 x 1.5 mL | L3000075 |
| Neon Transfection System | 1 unit | MPK5000 |
| QuantiGene Plex Assay | 1 plate | QP1013 |
| | 3 plates | QP1014 |
| | 10 plates | QP1015 |
| TrueCut Cas9 Protein v2 | 10 µg | A36496 |
| | 25 µg | A36497 |
| | 100 µg | A36498 |
| | 500 µg | A36499 |
| TrueGuide Synthetic gRNA, predefined | 3 nmol | A35510 |
| TrueGuide Synthetic gRNA, custom | 3 nmol | A35513 |
| Antibodies for Arginase-1 | thermofisher.com/antibodies | |
| Antibodies for FOXP3 | | |
| Antibodies for Granzyme B | | |
| Antibodies for LAG3 | | |
| Antibodies for TIGIT | | |
| Attune NxT Flow Cytometer | thermofisher.com/attune | |
| Attune CytPix Flow Cytometer | | |
| Bigfoot Spectral Cell Sorter | thermofisher.com/bigfoot | |
| Click-iT EdU Pacific Blue Flow Cytometry Assay Kit | 50 assays | C10418 |
| Click-iT EdU Alexa Fluor 647 Flow Cytometry Assay Kit | 50 assays | C10424 |
| Click-iT EdU Alexa Fluor 488 Flow Cytometry Assay Kit | 50 assays | C10425 |
| EVOS M7000 Imaging System | thermofisher.com/evos | |
| EVOS Onstage Incubator | | |
| EVOS M5000 Imaging System | | |
| EVOS FLoid Imaging System | | |
| EVOS XL Core Imaging System | | |

## Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|
| Human Plasma–Like Medium (HPLM) | 500 mL | A4899101 |
| | 10 x 500 mL | A4899102 |
| GeneArt Gene Synthesis Services | Custom | thermofisher.com/genesynthesis |
| GeneArt Gene-to-Protein Services | Custom | thermofisher.com/genetoprotein |
| PrimeFlow RNA Assays* | 40 assays | 88-18005-204 |
| | 100 assays | 88-18005-210 |
| Ion GeneStudio S5 System | 1 unit | A38194 |
| Oncomine TCR Beta-LR Assay | 1 each | A35386 |
| Oncomine TCR Beta-SR Assay | 1 each | A39072 |
| Oncomine Immune Response Research Assay | 30 assays | A32881 |
| Oncomine Tumor Mutation Load Assay | 24 assays | A37909 |
| Immuno-Oncology Checkpoint Markers Panel | 96 tests | EPX14A-15803-901 (Panel 1) |
| | 96 tests | EPX140-15815-901 (Panel 2) |
| CellInsight CX5 High Content Screening Platform | 1 each | CX51110 |
| CellInsight CX7 High Content Analysis Platform | 1 each | CX7A1110 |
| CellInsight CX7 LZR Pro High Content Screening Platform | 1 each | HCSDCX7LZRPRO |
| PureLink Expi Endotoxin-Free Maxi Plasmid Purification Kit | 4 preps | A33073 |
| | 10 preps | A31217 |
| | 25 preps | A31231 |
| Dynabeads Human T-Activator CD3/CD28 for T Cell Expansion and Activation | 0.4 mL | 11161D |
| | 2 mL | 11131D |
| | 5 x 2 mL | 11132D |
| Dynabeads Human T-Activator CD3/CD28/CD137 | 0.4 mL | 11162D |
| | 2 mL | 11163D |
| Dynabeads Human T-Expander CD3/CD28 | 10 mL | 11141D |
| CTS Rotea Counterflow Centrifugation System | thermofisher.com/rotea | |
| CTS Xenon Electroporation System | thermofisher.com/xenon | |

* Invitrogen™ PrimeFlow™ Probe Sets are needed separately for each target. Go to thermofisher.com/primeflow to view a complete listing of over 8,200 synthesized probe sets.

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