Immuno-oncology product resource guide

Your comprehensive guide to tools for immuno-oncology research

Thermo Fisher Scientific
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 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.
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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.
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, shown in 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 (shown in 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) that are taken from tumor tissue, modified in vitro, 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.
**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.

Cancer research

Key approaches

- **Checkpoint inhibition**
  Page 8—Find workflow tools for genomic biomarker discovery, verification, and protein expression, as well as immune checkpoint analysis by antibodies and assays.

- **CAR T cell therapy**
  Page 12—Find workflow tools for sequencing and cell engineering to T cell activation and expansion, including scalable solutions that help get you from basic research to the clinic.

- **Cancer vaccines**
  Page 16—Find transfection solutions, gene-to-protein services, and genomic biomarker tools to develop vaccine therapies.

**Biomarker discovery** — **Targets** — **Modification of model systems** — **Characterization and verification** — **Clinical immunotherapy**

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.

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

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 new Gibco™ ExpiSf™ Expression System, the first-ever chemically defined baculovirus expression system that can generate up to 3x more protein compared to existing 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 superior 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.

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.
Antibodies

- **Invitrogen™ antibodies**—Detect key checkpoint targets with primary, secondary, and custom validated antibodies.
  - Foxp3
  - Human TIGIT
  - Human LAG3 (and CD223, PD1, CTLA4, TIM3, LAG3, and VISTA)
  - Arginase-1 (and 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.

**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/custommabs]

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**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™ Anti-DR3 (TNFRSF25) ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 702277, 2 μg/mL) in conjunction with Invitrogen™ Alexa Fluor™ 488 Goat Anti–Rabbit IgG Superclonal™ 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.
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.

- **Invitrogen™ Click-iT™ EdU assays**—Provide a simplified, more robust assay for analyzing DNA replication in proliferating cells compared to traditional BrdU methods.

- **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.”

Dr. 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 industry-leading speed and scalability. With the Ion GeneStudio S5 Systems, researchers can select from five different chips to sequence 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 7, 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 Protein v2**—Get 2x higher editing efficiency in difficult targets (including immune cell lines) than with other suppliers’ products.

• **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 accuracy.

• **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.

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**Figure 7. 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](http://thermofisher.com/cartcelltherapy)
**T cell editing**

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

**Figure 8. 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.

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**Application note**

**Need to learn more about harnessing CRISPR technology for I-O research?**

You can achieve up to 90% functional knockout in human primary T cells.

Find out more at thermofisher.com/crisprprotein
Translate your cell therapy to the clinic with cGMP-grade reagents
Advancing your cell therapy product from research to clinical applications requires careful material selection and thoughtful process development. Our Gibco™ Cell Therapy Systems (CTS™) products 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.

Cell isolation and activation
- 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].

Cell 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™ 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 Gibco™ AIM V™ Medium.

Figure 9. T cell stimulation with Dynabeads™ T-Activator CD3/CD28. Human PBMCs were isolated from whole blood using a Ficoll™ separation. T cells coincubated with Dynabeads T-Activator CD3/CD28 showed proliferation from day 0 to day 5. Images were taken on an Invitrogen™ EVOS™ XL Core Imaging System at 40x magnification.
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, loss of heterozygosity (LOH) and copy-neutral LOH, chromothripsis, aneuploidy, and more to identify important copy number–based biomarkers.

Gene synthesis services
- **Invitrogen™ GeneArt™ Gene Synthesis**—Leverage industry-leading 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 10 shows an example of increased expression by optimized gene sequences in different host cells [16].

Find out more at [thermofisher.com/vaccineresearch](https://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 10-fold higher efficiency into the broadest spectrum of difficult-to-transfect cells.

- **Invitrogen™ Invivofectamine™ 3.0 Reagent**—Utilize a breakthrough reagent for in vivo RNAi delivery, with greatly improved performance and up to 85% knockdown achieved using microgram levels of siRNA.

- **Invitrogen™ mMESSAGE mMACHINE™ SP6 Transcription Kit**—Designed for in vitro synthesis of large amounts of capped RNA. Capped RNA mimics most eukaryotic mRNAs found in vivo, because it has a 7-methylguanosine cap structure at the 5’ end.

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**Figure 10.** The combination of GeneArt expression optimization and advanced Gibco expression systems (e.g., Expi293F cells) from Thermo Fisher Scientific 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].

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**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](http://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 the highest-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® 4 Fluorometer
- Thermo Scientific™ NanoDrop™ One/One® Microvolume UV-Vis Spectrophotometer

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

- Amplify target DNA
  - Applied Biosystems™ SimpliAmp®, Veriti®, and ProFlex® thermal cyclers
  - Applied Biosystems™ MicroAmp™ plastic consumables
  - Invitrogen™ custom DNA oligos
  - Invitrogen™ Platinum® Taq DNA Polymerase and Platinum® SuperFi™ DNA Polymerase

- Analyze data by electrophoresis
  - Invitrogen™ E-Gel® Power Snap 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™ AmpI Taq® 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 clean up
  - Applied Biosystems™ BigDye® X Terminator™ 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
Characterize and verify

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™ RNAlater™ Stabilization Solution
- Invitrogen™ RNaseZap™ RNA Decontamination Solution
- Invitrogen™ TRIzol™ Reagent for DNA, RNA, or protein isolation
- Invitrogen™ PureLink™ RNA purification kits
- Invitrogen™ mirVana™ miRNA isolation kits
- Applied Biosystems™ Arcturus™ LCM Instrument
- KingFisher purification systems for DNA and RNA with MagMAX nucleic acid purification kits
- Qubit 4 Fluorometer
- NanoDrop One/OneC spectrophotometers

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

- Synthesize and amplify cDNA
  - Invitrogen custom DNA oligos
  - Invitrogen™ Value Oligos
  - Invitrogen™ SuperScript™ IV One-Step RT-PCR System
  - SuperScript IV One-Step RT-PCR System with ezDNase™ Enzyme
  - MicroAmp plastic consumables
  - SimpliAmp, Veriti, 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

### 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
Solution spotlight: genetic analysis tools

**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**—This assay is rapidly becoming an independent predictor for patient stratification for response to immunotherapy. 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 FACS-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 superior informatics for accurate clonality and CDR3 TCR beta chain sequence assessment without interference from primer bias.

thermofisher.com/ngs-immunereresponse

**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 leading-edge 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
## Characterize and verify

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

<table>
<thead>
<tr>
<th>Observe</th>
<th>Count</th>
<th>Visualize</th>
<th>Analyze: Flow cytometry</th>
<th>Analyze: Quantitative imaging</th>
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<tr>
<td><strong>Compact, all-in-one, transmitted-light systems for easy cell, colony, and tissue identification and visualization, and stem cell colony picking</strong></td>
<td><strong>Accurate and automated counting, monitoring of fluorescent protein expression in cells, and viability studies of cells in just a few seconds</strong></td>
<td><strong>Multicolor fluorescence detection in cells and tissues, plate scanning, z-stacks, and time-lapse studies</strong></td>
<td><strong>Run multicolor samples faster and achieve greater resolution with Invitrogen™ acoustics-assisted focusing technology—with little fear of sample loss due to clogging</strong></td>
<td><strong>Integrate high-content screening and analysis into your workflow and analyze up to 500,000 phenotypic measurements in less than 5 minutes</strong></td>
</tr>
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- Invitrogen™ EVOS™ XL and XL Core Imaging Systems
- Invitrogen™ Click-it™ colorimetric IHC detection kits
- Invitrogen™ primary and secondary antibodies for IHC
- Thermo Scientific™ Nunc™ cell culture plastics
- Gibco™ cell culture media
- Invitrogen™ Countess™ II Automated Cell Counter
- Invitrogen™ Countess™ II FL Automated Cell Counter
- Countess II FL reusable slides
- Countess II disposable slides
- Invitrogen™ EVOS™ LED light cubes
- Invitrogen™ ReadyProbes™ reagents
- Invitrogen™ LIVE/DEAD™ reagents
- Invitrogen™ EVOS™ FL Imaging System
- Invitrogen™ EVOS™ FL Auto 2 Imaging System with optional Celleste™ Image Analysis Software
- Invitrogen™ EVOS™ Onstage Incubator
- Invitrogen primary and secondary antibodies for IHC
- Invitrogen Alexa Fluor and Alexa Fluor Plus antibodies
- Invitrogen™ fluorescent reagents
- Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader
- Thermo Scientific™ Nunc™ microwaves for cell-based assays
- Invitrogen™ Attune™ NxT Flow Cytometer
- Invitrogen™ Click-it™ EdU assays
- Invitrogen™ CellTrace™ cell proliferation kits
- Invitrogen™ Vybrant™ DyeCycle™ stains
- Invitrogen™ FIX & PERM™ Cell Permeabilization Kit
- Invitrogen™ Alexa Fluor™, Qdot™, and eBioscience™ Super Bright antibodies
- Invitrogen™ PrimeFlow™ RNA assays
- Thermo Scientific™ CellInsight™ CX6, CX7, and CX7 LZR HCS/HCA platforms
- Invitrogen™ HCS CellMask™, NuclearMask™, and CellTrace™ stains
- Invitrogen™ Alexa Fluor™ Plus secondary antibodies
- 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](http://thermofisher.com/mpsf)
Solution spotlight: EVOS FL Auto 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 (CO₂ and O₂) during an experiment was needed. The EVOS FL Auto Imaging System with Invitrogen™ 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.

Figure 11. A549 cells stained with Invitrogen™ Image-iT™ Hypoxia Reagent and exposed to different oxygen levels. (A) 20% O₂, (B) 5% O₂, (C) 2.5% O₂, and (D) 1% O₂.

Find out more at thermofisher.com/evos
Solution spotlight: Attune NxT Flow Cytometer

Advance I-O research with high-performance capabilities:

• Highest level of data fidelity featuring acquisition of 35,000 events/second and 1 mL/min 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.

Figure 12 shows an example of examining cells from tumor and blood samples with the Attune NxT Flow Cytometer. The volumetric fluidics and acoustics-assisted hydrodynamic focusing system of the Attune NxT Flow Cytometer produce highly accurate cell concentration data with an increased sampling rate.

Figure 12. Lymphocyte subset analysis. A 100 μL aliquot of normal human whole blood was labeled with fluorophore-conjugated antibodies against CD surface markers, followed by red blood cell lysis using 2 mL of Invitrogen™ High-Yield Lyse Fixative-Free Lysing Solution (Cat. No. HYL250), resulting in a 1:21 dilution of the blood. (A) Lymphocytes are identified on a density plot of CD45 vs. side scatter with an oval gate around the lymphocyte (CD45+ population). (B) Cells in the lymphocyte gate are displayed on a density plot of CD3 vs. side scatter. Rectangle gates surround the CD3+ T cell, and CD3- B and natural killer (NK) cell populations. (C) Cells in the CD3+ gate are then displayed on a density plot of CD8 vs. side scatter. Rectangle gates surround the CD8+ cytotoxic T cells (CD4+, CD8+, and CD45+), and CD8+ cytotoxic T cells (CD4+, CD3+, and CD45+). (D) CD3+ cells are displayed on a density plot of CD56 vs. CD19 to distinguish CD56+ NK cells from CD19+ B cells. The statistics table shows the gating and measured concentrations (cells/μL). (E) Replicate samples collected at three flow rates on the Attune NxT Flow Cytometer. Cell concentrations were measured using three different flow rates: 100, 200, and 500 μL/min. The Attune NxT Flow Cytometer provides similar concentration measurements for each lymphocyte subpopulation, regardless of the flow rate. Each bar represents the mean cells/μL ± standard deviation of three samples run at each indicated flow rate for each population.

Find out more at thermofisher.com/attune
Solution spotlight: CellInsight CX7 High Content Analysis 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, the CellInsight CX7 HCA Platform is used to detect cellular efficacy and off-target side effects of different CAR T cell therapies (Figure 13).

Figure 13. CAR T cell invasion into cancer spheroids. HCC827 spheroids were formed using a spheroid microplate for 48 hours. Twenty-four hours after addition of EGFR scFv-CD28-CD3ε CAR T cells (ProMab Biotechnologies), spheroids were stained for cytokeratin-7 (green) and CD3ε (red), with Hoechst nuclear counterstain (blue). As the effector-to-target (E/T) ratio is increased from 10:1 to 40:1, invasion of the CAR T cells into the HCC827 tumor spheroid and subsequent tumor cell lysis are visible. Images courtesy of Corning Inc., obtained using the CellInsight CX7 HCA Platform in confocal mode using 10x objective.

Find out more at thermofisher.com/cx7
## Protein analysis

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

<table>
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<th>Separate</th>
<th>Transfer</th>
<th>Detect</th>
<th>Quantity</th>
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<td>• Chromatography media&lt;br&gt;• Dialysis products&lt;br&gt;• Desalting products&lt;br&gt;• Concentrator devices&lt;br&gt;• Magnetic beads</td>
<td>• Precast protein gels&lt;br&gt;• Pour-your-own protein gel system&lt;br&gt;• Protein ladders&lt;br&gt;• Gel tanks</td>
<td>• Buffers&lt;br&gt;• Membranes&lt;br&gt;• Transfer systems</td>
<td>• Western blotting reagents and devices&lt;br&gt;• Chemiluminescent substrates&lt;br&gt;• Western blot imaging systems</td>
<td>• Total protein and immunoassays&lt;br&gt;• Mass spectrometry reagents for depletion, calibration, and labeling</td>
<td>• Protein crosslinkers&lt;br&gt;• Bioconjugation reagents</td>
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</table>

| • 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®, WedgeWell®, Bolt®, and NuPAGE® precast gels<br>• Invitrogen™ SureCast™ Gel Handcast System<br>• Thermo Scientific™ PageRuler® and Spectra™ protein ladders<br>• Invitrogen™ Mini Gel Tank | • 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™ 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>• Qubit fluorometers<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 |

### Realize the full potential of your protein studies

- Preserve your protein sample during extraction and purification
- Increase your western blotting throughput with our innovative benchtop systems
- Increase the reproducibility of your western blot experiments
- Get more information from your samples with our cutting-edge mass spectrometry reagents
### Ordering information

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*Immuno<sup>®</sup> PrimeFlow<sup>®</sup> 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.*
References