

Cancer research guidebook

Your comprehensive resource for cancer research solutions

Let nothing stand in the way of your next discovery

We understand the challenges cancer researchers face. Our goal is to support you with a comprehensive range of technologies and solutions that help you maximize your time, budget, data, and resources accelerating your path from discovery to clinical translation.

In this guidebook, you will discover educational resources and solutions for a number of challenges cancer researchers face. Learn about how to maximize funding, save time, save precious samples, and more. We're committed to partnering with you for your next breakthrough.



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Our commitment to cancer researchers

With over 100 subtypes, cancer is among the leading causes of death worldwide (Figure 1) [1,2]. We at Thermo Fisher Scientific understand that fighting cancer is more than your research it's your passion to advance science in the pursuit of better outcomes. Every factor in the service of this struggle is precious: from samples, technology, data, and budgets, to those who depend on your success. Every day, cancer continues to devastate lives. There's simply no room for waste or error.

Cancer research challenges

We spoke with many cancer researchers across the globe to understand how we can help make their journey easier. As part of that effort, we identified six common areas where we could help circumvent challenges:

- Time is too precious
- Funding is limited
- Samples are precious
- New techniques and education are needed
- Data are too limited
- Collaboration is limited

Because the obstacles are great and challenges are many, we must partner together to become more efficient, move faster, and evolve more quickly than this tenacious disease.



NCI predicts global cancer cases will increase by 50% from 2012 to 2030—that's a rise from 14 million cases to 21 million cases [2].



Our commitment to you:

• We will help shorten your path to breakthroughs by enabling you to maximize every resource: time, technology, money, samples, and data

NCREA

NCI predicts that by 2030

a rise from 8 million to

13 million deaths [2].

cancer deaths will increase

by 60% worldwide-that's

- We will be with you at every step, providing access to the tools, innovations, and support you need to empower your next discovery
- We will make the path to identifying the key technologies and resources we offer easier, to help you at every stage of your research journey

Together we can be smarter, more efficient, faster, and more determined than ever. The extraordinary talents and technologies of this generation promise to transform the future of cancer research. Let's begin a new era of collaboration in the fight against cancer.



Gain access to product bundles for your cancer research.*

Apply today at thermofisher.com/cancerresearchfoundation

* Terms and conditions apply. For complete details, go to thermofisher.com/cancerresearchfoundation

We want to help you during each stage of your research and will continue to maintain and update vital resources at thermofisher.com/cancerresearch



Time is too precious—scientists shouldn't have to spend excessive time creating, testing, and validating new protocols and workflows. New technologies have led to products that are designed to work together for rapid and reliable results. In this chapter, explore solutions that will free up more of your time for discovery.

Whether your cancer research would benefit from advanced technologies or workflow efficiencies, the time-saving products featured here could make all the difference in the speed of research.

Technology spotlight: Attune NxT and Attune CytPix Flow Cytometers for rapid cell analysis

Save time with the high-performance capabilities of the Invitrogen[™] Attune[™] Flow Cytometer family. These dynamic instruments are designed with active mechanical resistance to clogging, novel acoustics-assisted hydrodynamic focusing for speed, and sample acquisition from 96-well and 384-well microplates for reliable automation. Immunophenotyping analysis on minimally processed samples can be performed using a simplified no-wash, no-lyse method, reducing the time-intensive traditional sample preparation protocols from 10 steps to 3 straightforward steps.

The Attune CytPix model includes a high-speed brightfield camera that helps you visually confirm that your gates contain cells of interest and discover relevant morphology. The camera and Invitrogen[™] Attune[™] Cytometric Software help to ensure that the events you analyze are single cells as opposed to doublets, clumps, or debris. This is crucial in cell and gene therapy research applications, but is also useful in almost any flow cytometry experiment to help researchers understand the morphology of each cell population identified for analysis.

Figures 2 and 3 (on following pages) show example data from each instrument. The rapid imaging functionality of the Attune CytPix Flow Cytometer enables clear visualization of cell morphology, including cell-to-cell interactions. The volumetric fluidics and acoustics-assisted hydrodynamic focusing system of the Attune NxT Flow Cytometer produce highly accurate cell concentration data across sampling rates.



Straight from the scientist

"We don't get clogging, which for people who use cytometry, this is a problem we've just become used to, and with this instrument that's no longer an issue. The speed at which we can analyze using the Attune NxT Flow Cytometer is quite fast, and the efficiency by which we can identify these cells is extremely high. We get very tight, reliable data which is making us obviously more excited in the laboratory because we're actually now able to see this very small population of cells and believe the data that we are seeing."

-Bruno Sainz, PhD, Universidad Autónoma de Madrid

"With multiparameter capabilities and a very high analysis rate, flow cytometry is at present the most potent technology to address rare-cell analysis. When I first studied iNKT cell populations in HIV-positive patients, I had to deal with a more evident paucity of cells (iNKT cells are rarer in these samples, as HIV-positive people are immunocompromised). More than 20 million cells should be stained to find these rare populations among PBMCs. No instrument was able to acquire 20 million events. The Attune NxT Flow Cytometer made my project reliable."

-Sara De Biasi, PhD, University of Modena and Reggio Emilia





Figure 2. Photographic evidence of engineered cell potency acquired using the Attune CytPix Flow Cytometer. CAR T cells and Ramos cells, freshly labeled with the Invitrogen[™] CellTrace[™] Far Red kit and CellTrace Violet kit, respectively, 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 10⁵ 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.









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



Figure 3. Rapid lymphocyte subset analysis using the Attune NxT Flow Cytometer. 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 1:20 dilution of the blood sample. (A) Lymphocytes are identified on a density plot of CD45 vs. side scatter by gating 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⁺, 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 concentration measurements for each lymphocyte subpopulation, regardless of the flow rate. Each bar represents the mean concentration ± standard deviation of three samples run at each indicated flow rate for each population.

Technology spotlight: closed systems for cell therapy research and development

Cell therapy is a powerful tool designed to use the body's immune system to find and kill cancer cells. However, the cell therapy development and manufacturing process is often complex and labor-intensive, and involves many open processes that can impact regulatory compliance and lead to inconsistencies.

Closed, modular systems like the Gibco[™] CTS[™] Rotea[™] Counterflow Centrifugation System and Gibco[™] CTS[™] Xenon[™] Electroporation System enable you to use the same equipment from research through process development and manufacturing, thus eliminating the need to learn and optimize new systems. This helps cut process development times and delays, enabling you to reach your milestones faster while minimizing errors and contamination.

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



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 Invitrogen[™] 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: Invitrogen flow cytometry reagents

We offer a wide variety of Invitrogen[™] functional reagents to support cancer research and >13,000 primary antibody conjugates for flow cytometry. Measuring cell proliferation is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anticancer drugs. Invitrogen[™] Click-iT[™] EdU flow cytometry assays provide fast detection of DNA synthesis, in as little as 60 minutes, as an alternative to the cumbersome BrdU assay (Figure 4). Detection of cell populations can also be accomplished with Invitrogen[™] CellTrace[™] Cell Proliferation Kits that permanently label cells with fluorescent stains to trace generations or divisions without affecting morphology or physiology.

Achieve exceptional results from immunophenotyping with our highly referenced intracellular fixation and permeabilization buffers. Remove dead cells and unwanted artifacts during analysis using Invitrogen[™] LIVE/DEAD[™] fixable viability dyes, and correct for overlapping fluorescence emission spectra using Invitrogen[™] UltraComp eBeads[™] Compensation Beads. Invitrogen[™] Ready Flow[™] ready-to-use flow cytometry reagents provide time-saving, simplified protocols that do not require dilutions, calculations, or pipetting. These room temperature– stable reagents are available for most common needs, including dead cell identification, cell cycle analysis, and apoptosis.

Solution spotlight: iWestern workflow for western blot analysis

Leverage an inventive take on a decades-long problem: getting the desired results from what can be the elusive, time-consuming western blot. Designed to address processing efficiency, reproducibility, and robustness of results, the Invitrogen[™] iWestern[™] workflow enables exceptional western blotting results with minimal hands-on time. With the iWestern workflow, your life in the lab can become easier and less time-consuming, allowing you to focus on what matters most—your data. The iWestern workflow contains four innovative products designed for efficiency, reproducibility, and performance (Figures 5 and 6):

- Invitrogen[™] Mini Gel Tank
- Invitrogen[™] iBlot[™] 2 Gel Transfer Device
- Invitrogen[™] iBind[™] Western Device
- Invitrogen[™] iBright[™] Imaging System



Figure 5. Streamline the western blotting process with products in the iWestern workflow. From left to right: Mini Gel Tank, iBind Western Device, iBlot 2 Gel Transfer Device, and iBright Imaging System.



Figure 4. Dual-parameter plot of human T cells from a time-saving assay for CD3 and DNA strand breaks. In less than 90 minutes, the Invitrogen[™] Click-iT[™] Plus EdU Alexa Fluor[™] 488 Flow Cytometry Assay Kit detected DNA strand breaks, saving multiple steps and 5 hours or more of work as compared to using the traditional BrdU detection technique. DNA strand break detection was fully compatible with detection of CD3 with a PE-Cy®7–labeled anti-CD3 antibody.

Straight from the scientist

"I highly recommend the iBright system. I truly enjoy the membrane overlay option as well for my prestained ladder. It is extremely user friendly while producing sharp, clear images. I also love the fact that the iBright system can export publicationquality images straight to the Thermo Fisher[™] Cloud Platform that you can then access from anywhere!"

-Amanda Eccardt, PhD candidate, Saint Louis University

Step	iWestern workflow	Manual workflow
Sample prep/gel electrophoresis	50 min	90 min
Transfer	7 min	75 min
Blocking		60 min
Primary incubation		720 min
Washes	180 min	60 min
Secondary incubation		60 min
Substrate incubation	5 min	5 min
Target detection	2 min	5 min
Total time	244 min (~4 hr)	1,075 min (~18 hr)

Figure 6. Comparison of data obtained using the iWestern workflow and manual western blotting. The iWestern workflow enables the detection of HDAC1 in HeLa lysate in approximately 4 hours, vs. 18 hours using the manual western workflow. Two-fold serial dilutions (starting with 20 µg of HeLa lysate) were loaded into wells of Tris-glycine gels, and western blotting was performed on nitrocellulose membranes using each procedure. The target was detected using the Thermo Scientific[™] SuperSignal[™] West Pico PLUS Chemiluminescent Substrate.

Instant ELISA kits

Make the ELISA process easier and more reliable, reducing the typical workflow by 30% while maintaining the performance you require. With Invitrogen[™] Instant ELISA[™] kits, there is no laborious preparation of reagents, serial dilutions of standards, or sequential additions to the plate. These kits are an excellent option for labs requiring a streamlined ELISA system with maximum accuracy, reproducibility, and time savings (Figures 7 and 8).



Figure 7. Performance comparison of Instant ELISA kit vs. coated ELISA kit. The Invitrogen[™] CD44v5 (Soluble) Human Instant ELISA[™] Kit (Cat. No. BMS220INST) was tested against the Invitrogen[™] CD44v5 (Soluble) Human ELISA Kit (Cat. No. BMS220). Sixteen serum samples were tested with each kit, demonstrating comparable performance of the kits with a coefficient of determination (r²) of 0.98.



Figure 8. Comparison of Instant ELISA technology vs. conventional ELISA procedures. With Instant ELISA kits, all the preparation work has been done for you, thereby decreasing hands-on time per experiment. Instant ELISA kits contain all of the necessary components, including capture antibody and lyophilized detection antibody, streptavidin-HRP, sample diluent, and ready-to-use strip wells for the standard curve.

Microplate washers and spectrophotometers

Pairing the Thermo Scientific[™] Wellwash[™] Microplate Washer with the Thermo Scientific[™] Multiskan[™] SkyHigh Microplate Spectrophotometer and Skanlt[™] Software make washing, detection, and analysis during the ELISA workflow easier and more efficient. Experiments are straightforward to set up and flexible for modifications, like onboard shaking and incubation and easy selection of wavelengths. The Wellwash Microplate Washer automates the critical wash steps necessary during ELISAs. Incomplete washing will adversely affect the results of most ELISAs. SkanIt Software offers fast, simplified data acquisition and analysis with features such as versatile curve fit options, sample classification, and built-in quality control tools.

Find out how to create a complementary platform for your ELISA experiments at <u>thermofisher.com/platereaders</u>



E-Gel electrophoresis systems and precast gels

Combine the convenience of rapid, real-time nucleic acid analysis with the precision of high-resolution image capture to reduce workflow time and accelerate discovery. Using the Invitrogen[™] E-Gel[™] Power Snap Plus Electrophoresis System for high throughput or the E-Gel Power Snap Electrophoresis System for low throughput with precast E-Gel agarose gels can simplify the nucleic acid electrophoresis workflow (Figure 9). There are no gels to pour, buffers to make, staining or destaining steps to perform, or gel boxes to assemble. Just load your samples and run.



Figure 9. Simplified workflow for nucleic acid analysis that helps save time. Both electrophoresis systems along with precast gels offer excellent sample resolution in as little as 15 minutes and are ideal for quickly analyzing PCR products, restriction digests, plasmid preparations, and genotyping products.

Platinum II Taq Hot-Start DNA Polymerase

Achieve faster, more efficient PCR with a unique universal primer annealing feature that allows for co-cycling of all assays, reducing tedious optimization steps. Invitrogen[™] Platinum[™] II *Taq* Hot-Start DNA Polymerase has also been engineered to synthesize DNA up to 4x faster than standard *Taq* polymerase, reducing PCR run times compared to products from other suppliers (Figure 10).



Figure 10. Fast cycling reduces PCR run time. Amplification of a 529 bp fragment from 50 ng of human genomic DNA in 50 µL reactions for 35 cycles was carried out using Platinum II *Taq* Hot-Start DNA Polymerase and hot-start DNA polymerases from other suppliers: (A) Sigma-Aldrich KAPA2G[™] Fast HotStart PCR Kit, (B) NEB OneTaq[™] Hot Start DNA Polymerase, (C) Promega GoTaq[™] G2 DNA Polymerase, (D) Toyobo Quick *Taq[™]* HS DyeMix, (E) Roche FastStart[™] *Taq* DNA Polymerase, and (F) Sigma-Aldrich JumpStart[™] *Taq* DNA Polymerase. Cycling times for each polymerase are shown in teal, while ramping times on the Applied Biosystems[™] ProFlex[™] PCR System (6°C/sec peak block ramp rate) are shown in red. PCR product analysis in 1% TAE agarose gels is presented below the graph. The size marker is the Thermo Scientific[™] ZipRuler[™] Express DNA Ladder 2.

KingFisher Apex Purification System for DNA, RNA, protein, and cell extraction and isolation

KingFisher instruments automate extraction of DNA, RNA, protein, and cells by moving magnetic beads (not liquids), leading to clean extractions and enabling consistent results. Using a simple process—bind, wash, elute—KingFisher instruments can automate the extraction of any analyte of interest that is attached to a bead. The KingFisher Apex system (Figure 11) is available with four different user-interchangeable magnetic head formats, enabling processing of a variety of volumes with compatible purification reagents such as Applied Biosystems[™] MagMAX[™] nucleic acid extraction products.



Figure 11. The KingFisher Apex Purification System minimizes the hands-on time required for DNA, RNA, and protein isolation.

Dynabeads magnetic beads for protein isolation and immunoprecipitation

Invitrogen[™] Dynabeads[™] magnetic beads are the most cited products for sensitive applications such as chromatin immunoprecipitation (ChIP) and immunoprecipitation (IP) of low-abundance proteins. These highly uniform particles (Figure 12) reduce nonspecific binding and enable IP protocols to be performed in less than 40 minutes. Automated IP protocols are now available for use of Dynabeads products with KingFisher systems.



Figure 12. The magnetic bead you choose can help you save time.
Dynabeads magnetic beads have a defined surface to carry out the necessary binding, with no inner surface to trap unwanted proteins.
(A) Dynabeads products are the most uniform monodisperse superparamagnetic beads, manufactured with highly controlled product qualities to help ensure the highest degree of reproducibility. (B–D)
Magnetic particles from alternative suppliers have variable shapes and sizes that trap impurities, resulting in lower reproducibility and increased nonspecific binding.

Key product listing

Discover even more time-saving solutions with the products described here.



ProQuantum high-sensitivity immunoassays Invitrogen[™] ProQuantum[™] immunoassay kits enable

highly sensitive measurements of specific targets

such as IFNy, IL-8, and IL-6 using only a fraction of sample volume and with greater dynamic range compared to traditional methods. These assays offer a simplified workflow with analysis using qPCR technology.



Cancer research primary antibodies and **ELISA** kits

Find the antibodies you need with our highly validated* portfolio. We have more than 200,000 antibodies to choose from, covering more than 85% of the human genome. Or search our ready-to-use, tested and validated ELISA kits for cancer research. Use our simple web search tool to find the antibody (thermofisher.com/antibodies) or ELISA (thermofisher.com/elisa) that you need. To help ensure that you achieve superior results, we are redefining our approach to antibody specificity testing. In addition to application testing methods, we are expanding validation to include advanced verification methods such as knockout/knockdown. IP-MS. SNAP-ChIP[™] assays, and others.



Oncomine cell-free assays

Ion Torrent[™] Oncomine[™] cell-free nucleic acid assays are multibiomarker NGS assays that enable reproducible detection of somatic mutations in plasma, down to a level of 0.1% in genes relevant to solid tumors. These assays are designed to use a single tube of blood for analysis of SNVs, short indels, CNVs, and fusions that are frequently mutated in cancer research samples. When used as part of a complete NGS workflow, the process from blood research sample to variant data takes as little as 2 days.



Ion GeneStudio S5 series

Semiconductor sequencing instruments enable the production of high-quality sequencing data in just a

few hours. With the Ion GeneStudio[™] S5 series, you can go from DNA library to data in as little as 24 hours, with only 45 minutes of hands-on time, when paired with the Ion Chef[™] System.

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic use.

S Purchase with confidence

Reliable tools and reagents help cancer researchers achieve more with limited funding. From antibodies with demonstrated performance to tools that work better together, whole-workflow procurement is now possible at Thermo Fisher Scientific. Enjoy more consistent quality, decrease your purchasing workload, and simplify troubleshooting. Start saving with smart solutions—the following core reagents for cancer research may be your biggest opportunity to achieve cost efficiency.

Antibodies verified for performance

Invitrogen[™] antibodies are tested using a variety of methods to help ensure the highest levels of confidence. In the following examples, EGFR protein is measured in control cells and in cells in which the target gene has been knocked out using CRISPR-Cas9 technology. The lack of binding by EGFR antibody after elimination of EGFR protein expression demonstrates that the antibody is specific (Figures 13 and 14).

Straight from the scientist

"Up to US \$800 million per year are wasted on poorly characterized or ill-defined antibodies." [5]



Figure 13. Verification of antibody specificity by western blot. (A) Western blot analysis of EGFR was performed on whole-cell extracts of A-431 control cells and A-431 EGFR knockout (KO) cells. EGFR was detected using Invitrogen[™] EGFR Monoclonal Antibody (Cat. No. MA5-13269). (B) Densitometric analysis of the western blot shows the absence of signal in the CRISPR-mediated knockout, which confirms that the antibody is specific to EGFR.





Figure 14. Verification of antibody specificity by immunofluorescence. EGFR expression was assessed using 70% confluent log-phase A-431 cells (wild type, panels a, d), Cas9 control cells (panels b, e), and EGFR knockout cells (panels c, f). The cells were fixed, permeabilized, and labeled with EGFR Monoclonal Antibody (Cat. No. MA5-13269, 5 µg/mL), followed by Invitrogen[™] Goat Anti–Mouse IgG (H+L) Superclonal[™] Recombinant Secondary Antibody, Alexa Fluor[™] 488 conjugate (Cat. No. A28175, 1:2,000). Nuclei (blue) were stained with Invitrogen[™] SlowFade[™] Gold Antifade Mountant with DAPI (Cat. No. S36938), and Invitrogen[™] Rhodamine Phalloidin (Cat. No. R415, 1:300) was used for cytoskeletal F-actin (red) staining. Loss of signal was observed in EGFR knockout cells (panels c, f) confirming specificity of the antibody to EGFR (green). The images were captured at 60x magnification.

Commitment to antibody specificity and reproducibility

Antibodies are some of the most critical research reagents used in the lab. Poor specificity or application performance can significantly hinder your ability to obtain good results, which can cause critical delays.

Thermo Fisher Scientific is working to redefine antibody performance standards with a comprehensive approach to how antibodies are evaluated and validated.* Specificity testing is combined with extensive application validation data to provide confidence that our high-quality Invitrogen antibodies will help enable superior performance in your research.

Standard antibody validation

Testing to help ensure the antibody works in particular applications of interest, which may include but are not limited to:

•

Western blotting
 IHC

•

•

- ICC and IF
- Flow cytometry

Advanced Verification

Testing to help ensure the antibody specifically binds the correct target in the applications of interest, which may include:

- Knockout
- Knockdown
- Independent antibody validation
- Cell treatment
- Relative expression
- NeutralizationPeptide array

ELISA

ChIP

IP

- SNAP-ChIP assay
- Immunoprecipitation-mass spectrometry (IP-MS)

Advanced Verification You can easily identify antibodies that have already undergone this additional testing with the Advanced Verification badge. This badge can be found in antibody search results and on appropriate antibody product webpages. The additional data supporting the Advanced Verification status can be found in the product-specific data image galleries.

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic use.

Primary antibodies

Antibodies are among the most critical reagents used in the lab, so using antibodies that perform from the start can help save time and money. Whether your research is focused on metastasis, proliferative signaling, apoptosis, autophagy, metabolism, inflammation, tumor suppressors, tumor immunology, or any other cancer-related research area, we have a broad selection of research antibodies to help ensure your success.

MAPK signaling pathway antibodies

The MAPK signaling pathway is essential in regulating many cellular processes, including inflammation, cell differentiation, cell proliferation, and death. There are at least three distinct signaling modules, including ERK, JNK, and p38 kinase.

AKT signaling pathway antibodies

AKT activation serves as a master switch for broadly influential cellular signaling pathways, generating a multitude of intracellular responses through a plethora of downstream targets and interacting partners. Use antibodies to examine key targets, including AKT1, AKT2, AKT3, and PI3K.

Phosphospecific antibodies

Our phosphospecific antibodies can be used in a wide variety of immunodetection applications, including western blot, immunohistochemistry, quantitative ELISA, and flow cytometry. Key targets include SRC, FAK, TAU, EIF2, AKT, ERK, JAK, STAT, and MAPK.

Transcription-specific antibodies

We offer a wide selection of specific primary antibodies for studying transcription factors. Key targets include CREB, c-Myc, c-Fos, AP2, AID, CDX2, ETS1, E2F1, and 4EBP1.

GTPase antibodies

GTP hydrolases (GTPases) cleave GTP. The GTPase family includes the RAS superfamily, which is critical to cell signaling. Key targets include RAC, RAS, ARF, RHO, RAB, and CDC42.

Recombinant antibodies

We offer primary and secondary antibodies that are produced using *in vitro* cloning and are widely considered to be the most specific and sensitive compared to other types of antibodies. Spend less time optimizing your studies and worrying about lotto-lot consistency.

Secondary antibodies

Cancer researchers can't afford to take risks with challenging samples for critical imaging or western blotting experiments. Invitrogen[™] Alexa Fluor[™] Plus secondary antibodies represent an advancement in fluorescent-conjugate technology, designed to provide brighter signals, enhanced sensitivity, and minimal cross-reactivity in a variety of samples and applications (Figure 15). Make your low-abundance targets visible, spend less time optimizing, and make every one of your precious samples count with antibodies that you can trust.



Figure 15. Alexa Fluor Plus secondary antibodies and antibody fragments demonstrate low cross-reactivity and deep tissue penetration during immunofluorescence analysis. (A) Human colon FFPE tissue sections were deparaffinized through a series of xylene and graded ethanol washes with a final wash in ddH₂O. Samples were stained using Invitrogen[™] F(ab')₂ Goat Anti–Mouse IgG Alexa Fluor[™] Plus 594 secondary antibody (Cat. No. A48288), F(ab')₂ Goat Anti–Rabbit IgG Alexa Fluor[™] Plus 555 secondary antibody (Cat. No. A48283), and other Invitrogen[™] reagents. (B) Sprague-Dawley rat intestine (duodenum frozen sections) was stained using F(ab')₂ Goat Anti–Rabbit IgG Alexa Fluor Plus 555 secondary antibody (Cat. No. A48283) and other Invitrogen reagents. (C) Human colon FFPE tissue sections were deparaffinized through a series of xylene and graded ethanol washes with a final wash in ddH₂O. Samples were stained with Invitrogen[™] F(ab')₂ Goat Anti–Rabbit IgG Alexa Fluor[™] Plus 488 secondary antibody, F(ab')₂ Goat Anti–Mouse IgG Alexa Fluor Plus 594 secondary antibody (Cat. No. A48288), and other Invitrogen reagents. (D) BPAE cells were stained with Invitrogen[™] F(ab')₂ Goat Anti–Rabbit IgG Alexa Fluor[™] Plus 495 secondary antibody (Cat. No. A48287) and other Invitrogen reagents. (D) BPAE cells were stained with Invitrogen[™] F(ab')₂ Goat Anti–Rabbit IgG Alexa Fluor[™] Plus 495 secondary antibody (Cat. No. A48287) and other Invitrogen reagents.

ProQuantum high-sensitivity immunoassays

Leverage a new platform that enables measurement of targetspecific proteins such as IFNy, IL-8, and IL-6. Invitrogen™ ProQuantum[™] immunoassay kits utilize proximity ligation assay (PLA[™]) technology and 2 µL of sample or less per run (Figure 16). Unlike other next-generation immunoassay platforms on the market, you do not need to buy an additional, closed system. ProQuantum immunoassays can be run on any existing gPCR instrument, which maximizes the value of the instruments you already have.



Figure 16. ProQuantum immunoassays help reduce costs by utilizing widely available qPCR instruments. PLA technology is used to combine antigen-antibody binding for analyte detection with qPCR signal amplification and readout. The assay is a two-stage process: (A) Two antibody conjugates are provided in each kit: a 3'-end oligonucleotide and a 5'-end oligonucleotide, each conjugated to a target-specific antibody. When the antibody pair binds to two different epitopes of the protein target, the 3' and 5' oligos come into close proximity. (B) Ligation of the oligonucleotides occurs by addition of DNA ligase and a splint oligonucleotide. Following ligation, 95°C heat inactivation denatures the ligase, antibodies, and other proteins, leaving 100-base strands in concentrations proportional to the level of antibody-analyte binding in the first stage. This 100-base DNA strand serves as the amplification template for 40 cycles of qPCR using Applied Biosystems[™] TaqMan[™] Assays.



Gibco media and Nunc plastics

Benefit from a proven combination of Gibco[™] cell culture media and Thermo Scientific[™] Nunc[™] plastics for consistent cell growth. Every lot of Nunc plastics with the Nunclon[™] Delta cell culture-treated surface is validated with trusted Gibco media to confirm consistent cell growth across multiple cell lines. Only Thermo Scientific[™] cell culture plastics endure rigorous testing with Gibco media, including cloning efficiency tests using specially selected cell lines with high sensitivity to toxic components. It's the perfect pairing that can mean the difference between success and starting over.

Key product listing

Budget maximizers come in all shapes and sizes. Explore the additional solutions described here.



Attune NxT Flow Cytometer

Customize experiments with up to 4 lasers and 14 colors with options for up to 6 channels off of the violet laser. Get the freedom to build multicolor panels,

accommodating common fluorophores and dyes.



Uncoated ELISA kits

Invitrogen[™] uncoated ELISA kits are ideal for experienced ELISA users on a budget, with less stringent requirements for inter- and intra-assay variance. These kits are several times more economical than ready-to-use ELISA kits that contain precoated plates.

ProFlex PCR system

Backed by the proven reliability of Applied Biosystems[™] technology, the Applied Biosystems[™] ProFlex[™] PCR System has 5 different blocks that can be changed with the flip of a switch, including the first-of-its-kind 3 x 32-well block. This block allows up to three experiments to be run simultaneously, completely independently of each other and without interference.

Efficiencies for precious samples

We're committed to helping you maximize every precious research sample. Feel reassured by tools that provide enhanced sensitivity so that you can accelerate your research path forward. From reagents to kits to instruments, our solutions are designed to empower your next discovery.

The products featured in this chapter can help you make the most out of your limited research samples.

Ion GeneStudio S5 System for NGS analysis

With simple cartridge-loaded reagents and a straightforward user interface, the Ion GeneStudio[™] S5 System makes NGS fast and easy—ideal for any cancer research lab (Figure 17). Minimize sample input and maximize flexibility. Ion AmpliSeq[™] technology enables successful library preparation from challenging sample types such as formalin-fixed, paraffin-embedded (FFPE) tissue, retrospective samples from fine-needle aspirates, and cell-free DNA (cfDNA) extracted from blood, using as little as 1 ng of input DNA or RNA to support multiple applications. With this system, you can efficiently implement small and large projects across multiple research applications with a simple sample-to-data NGS workflow.



Figure 17. The Ion GeneStudio S5 System requires minimal sample input to efficiently support multiple research applications in the NGS workflow.



Qubit Fluorometers for nucleic acid quantitation

Invitrogen[™] Qubit[™] Fluorometers are designed to quickly and specifically quantitate DNA or RNA. In addition, you can now easily measure RNA integrity and quality with the Invitrogen[™] Qubit[™] RNA IQ Assay Kit. It is now easier than ever to make sure you have enough DNA or RNA for your experiment. Use as little as 1 µL of sample, even with very dilute samples. Dyes in Invitrogen[™] Qubit[™] assays bind selectively to DNA, RNA, or protein, making them more sensitive than UV absorbance (Figure 18).



Figure 18. Comparison of quantifiable sample concentration ranges for Qubit assays vs. UV absorbance measurements. UV absorbance readings are not selective for RNA or DNA. HS: high sensitivity, BR: broad range, XR: extended range.

MagMAX Cell-Free DNA Isolation Kit

Isolating and analyzing circulating cell-free DNA (cfDNA) from plasma, serum, or urine has the potential to serve as a noninvasive approach to detect and monitor targets associated with certain disease states such as cancer. The Applied Biosystems[™] MagMAX[™] Cell-Free DNA Isolation Kit is designed for both manual and automated isolation of cfDNA from a wide sample volume input range (0.5 mL to 10 mL). The phenol-free, magnetic bead format makes the kit fully amenable to automated isolation, and the extracted DNA is ready for downstream analysis using real-time PCR, digital PCR, or next-generation sequencing. Use this kit with KingFisher instruments and Oncomine assays to optimize the liquid biopsy workflow from extraction and purification to analysis (Figure 19).

MagMAX Cell-Free Total Nucleic Acid Kit

In cases where circulating cell-free RNA (cfRNA) or circulating cell-free total nucleic acid (cfTNA) is required, the Applied Biosystems[™] MagMAX[™] Cell-Free Total Nucleic Acid Kit is the optimal solution. Designed to purify free-circulating DNA, RNA, and miRNA with a flexible sample input range of 1.0 to 6 mL, this magnetic bead–based kit also offers phenol-free extraction for both manual and automated workflows. The concentrated nucleic acid is ready for downstream analysis using real-time PCR, digital PCR, or next-generation sequencing. This kit can also be coupled with KingFisher instruments and Oncomine assays to create an optimal end-to-end liquid biopsy workflow (Figure 19).



Figure 19. A sample-to-answer liquid biopsy workflow.

MagMAX FFPE DNA/RNA Ultra Kit

Unlocking the information contained in FFPE samples is essential to cancer research. The Applied Biosystems[™] MagMAX[™] FFPE DNA/RNA Ultra Kit is designed for sequential isolation of DNA and RNA from the same FFPE tissue sample, with a minimal requirement of a 5 µm section or curl. The DNA and RNA are recovered in separate eluates, and both are compatible with a broad range of applications, including real-time PCR and NGS (Figure 20).

SuperScript IV VILO Master Mix

When working with challenging RNA samples or analyzing lowabundance transcripts, the ability of a reverse transcriptase to generate cDNA from the lowest amount of input RNA is crucial. InvitrogenTM SuperScriptTM IV VILOTM Master Mix offers highly sensitive and robust cDNA synthesis for RT-qPCR applications in a short 10-minute reaction. Obtain higher cDNA yields and earlier C_t values (up to 6 cycles ahead of other reverse transcriptases, Figure 21), all while maintaining excellent linearity across a broad range of RNA inputs. Additionally, outstanding inhibitor resistance allows for the utmost confidence in results, even with degraded or inhibitor-containing RNA samples.



Figure 20. Efficient real-time PCR analysis of DNA and RNA samples. (A) Comparable or mostly better C_t values were achieved with FFPE DNA samples compared to fresh-frozen (FF) samples. (B) Differences of 0.7–5 cycles in C_t were observed in the RNA samples.



Figure 21. Highest reverse transcription efficiency across a broad range of targets. RT-qPCR was performed with different cDNA synthesis reagents and Applied BiosystemsTM TaqManTM Assays. Delta C_t values ($\Delta C_t = C_t - C_t$ (SuperScript IV VILO Master Mix) show that SuperScript IV VILO Master Mix delivered C, values that were 2 cycles lower, on average, than those of the other reagents tested.

Technology spotlight: NGS tools for maximizing precious samples Oncomine TCR Beta-LR Assay

The Ion Torrent[™] Oncomine[™] TCR Beta-LR Assay is designed to efficiently capture all three complementarity determining regions of the TCR beta chain gene (CDR1, CDR2, CDR3) with as little as 10 ng of RNA input. 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 use of RNA template allows sequencing of productive and relevant variable (V), diversity (D), and joining (J) rearrangements—improving the identification of rare clones.

Oncomine TCR Beta-SR Assay

Requiring low sample input, the Ion Torrent[™] Oncomine[™] TCR Beta-SR Assay specifically interrogates the CDR3 region of the TCR beta chain gene. Compatible with both FFPE DNA and RNA, this short-read sequencing assay enables characterization of immune status and detection of T cell clones in peripheral blood. The assay offers a 2-day turnaround time and is complete with high-quality informatics for accurate clonality and sequence assessment of the CDR3 region without interference from primer bias.

Oncomine Tumor Mutation Load Assay

Tumor mutational burden (TMB) is rapidly becoming an independent predictor for patient stratification for response to immunotherapy. With a 3-day workflow using as little as 20 ng of tumor DNA, the Ion Torrent[™] Oncomine[™] Tumor Mutation Load Assay covers 1.65 Mb across 409 oncogenes relevant to major cancer types. The assay highly correlates with exome mutation counts and obviates the need for whole-exome sequencing, allowing for a higher percentage of samples to be evaluated and conserving precious samples for additional biomarker assessment. Additionally, our Oncomine informatics enable simultaneous assessment of TMB and variant profiling for greater actionable insights.

Key product listing

Maximize your samples even further with the solutions described here.



Arcturus Cellect Laser Capture Microdissection (LCM) System

The Applied Biosystems[™] Arcturus[™] Cellect LCM System is optimized for applications ranging from single-cell isolation to extraction of small cell populations with a high degree of precision and flexibility. The instrument enables researchers to uncover unique molecular signatures that may be obscured in heterogeneous tumor samples. This system features the unique combination of a gentle infrared (IR) laser to capture the cells of interest and a powerful ultraviolet (UV) laser for efficient microdissection.



Clariom D Pico and Clariom S Pico microarray assays

Many expression profiling techniques require large amounts of input RNA, which are often not available

in cancer research samples. Applied Biosystems[™] Clariom[™] Pico assays can generate detailed, robust, and accurate data from as little as 100 pg of total RNA (as few as 10 cells) from a wide range of challenging clinical research samples.



TagMan Mutation Detection Assays

Applied Biosystems[™] TaqMan[™] Mutation Detection Assays are powered by allele-specific TagMan PCR (castPCR[™]) technology to detect and measure somatic mutations in genes of interest to cancer research. This highly specific and sensitive technology can detect rare copies of mutated DNA in a sample that contains large amounts of wild type DNAdown to 1 cancer cell in 1,000 normal cells. These assays are compatible with sample types such as cell lines, FFPE tissue, and fresh-frozen tissue.

TagMan Advanced miRNA Assays

Applied Biosystems[™] TagMan[™] Advanced miRNA Assays enable highly sensitive and specific quantitation of mature miRNAs using gPCR. Together with the Applied Biosystems[™] TaqMan[™] Advanced miRNA cDNA Synthesis Kit, this solution provides a streamlined workflow that allows for the detection and quantitation of mature miRNA from as little as 1 pg of total RNA or 2 µL of plasma or serum.



SuperScript IV Reverse Transcriptase

Invitrogen[™] SuperScript[™] IV Reverse Transcriptase offers superb cDNA synthesis in a short 10-minute reaction. It is designed to provide reliable and consistent results even with the most challenging RNA samples.



Platinum SuperFi DNA Polymerase

Invitrogen[™] Platinum[™] SuperFi[™] DNA Polymerase is a proofreading enzyme that combines exceptional fidelity

with trusted Platinum[™] hot-start technology. Featuring >100x the fidelity of Tag polymerase, Platinum SuperFi DNA Polymerase is ideally suited for applications that benefit from exceptional sequence accuracy. In addition, the extreme robustness of the polymerase enables successful amplification of samples of suboptimal purity.



PureLink RNA Micro Scale Kit

The Invitrogen[™] PureLink[™] RNA Micro Scale Kit is designed to be used when input is limited. It provides rapid purification and concentration of total RNA from a variety of samples, including LCM tissues, fine-needle aspirates, and sorted cells.



SYBR Gold Nucleic Acid Gel Stain

Invitrogen[™] SYBR[™] Gold Nucleic Acid Gel Stain is the most sensitive fluorescent stain we offer for nucleic acid detection. Maximize your precious DNA and RNA samples with enhanced sensitivity to detect as little as 25 pg of sample.



SuperSignal West Femto Maximum Sensitivity Substrate

Thermo Scientific[™] SuperSignal[™] West Femto Maximum Sensitivity Substrate is an ultrasensitive enhanced chemiluminescent (ECL) substrate for low-femtogram detection of protein by western blot analysis with the enzyme horseradish peroxidase (HRP). This substrate produces a quantitative signal that is easily captured by exposure to film or with an imaging system. The substrate offers excellent signal duration-8 hours of usable light output when conditions are optimized.



Bolt Bis-Tris Plus mini gels

Invitrogen[™] Bolt[™] Bis-Tris Plus mini gels are designed for optimal separation of a broad molecular weight range of proteins under denaturing conditions during gel electrophoresis. The unique WedgeWell[™] design allows loading of up to two times more sample volume than other precast gels. Bolt gels are ideal for western blot analysis and applications where protein integrity is crucial and higher sensitivity is required.



CytoVista Tissue Clearing Reagent

Invitrogen[™] CytoVista[™] Tissue Clearing Reagent sufficiently clears tissue for 3D fluorescent imaging, but unlike other clearing techniques does not significantly change the tissue morphology. Minimal morphological changes, such as shrinkage and contraction, have been observed. For precious samples, the clearing can be reversed, and the tissue can be processed for histology applications such as hematoxylin and eosin (H&E) staining.

ECM products

Gibco[™] extracellular matrix (ECM) products are essential tools for the growth, differentiation, and maturation of 2D and 3D cellular models. Products including Gibco[™] Geltrex[™] matrix, collagen, and the AlgiMatrix[™] system are widely used to provide cells with more physiologically relevant environments that promote in vivo-like morphology. Lot-to-lot consistency and functional testing help provide increased confidence for specialized cell types like stem cells or cancer cell lines.

Advanced techniques made accessible

Techniques for gene manipulation, expression analysis, and cancer cell modeling can be difficult to implement in the lab. New technologies can help simplify these techniques and make them accessible to all researchers. This increased accessibility leads to broader adoption by the research community, allowing the techniques to be standardized over time.

From nucleic acids to proteins to cells, empower your cancer research workflow with the products and techniques featured in this chapter.

Synthetic biology spotlight

Our synthetic biology solutions, from Invitrogen[™] GeneArt[™] Gene Synthesis to CRISPR-Cas9 genome editing products, are helping scientists push the boundaries of cancer research. Sequencing and analyzing cancer-related genes, combined with the ability to specifically edit those genes with widely adopted CRISPR technology, will enable future discoveries.

Gene synthesis services

With Invitrogen[™] GeneArt[™] services, leverage outstanding sequence optimization and gene synthesis services, or outsource the entire process of protein production. Maximize protein yield with Invitrogen[™] GeneOptimizer[™] software that generates sequence variants with enhanced mRNA stability and translational efficiency. Figure 22 shows an example of increased expression with optimized gene sequences in different host cells [4].



Straight from the scientist

"Keeping up with the new technologies like genome engineering, TCR manipulating, and CRISPR reagents is important to us. We have been pretty focused on the core set of technologies, but with an eye toward the future, we do dip our toes in newer technologies—however, these are all exploratory at this point. But then again, having a partner like GeneArt [Gene Synthesis] that brings the newer technologies to us and helps us to do something new in a quick time frame is quite valuable to us."

-Peter Ebert, PhD, Adaptive Biotechnologies



Figure 22. Advanced gene synthesis techniques enhance protein expression.

The combination of GeneOptimizer software for sequence-optimized synthetic genes and advanced Gibco[™] expression systems (e.g., Expi293[™] system) usually leads to higher overall expression yields than can be obtained with nonoptimized genes. With the GeneOptimizer algorithm, common pain points associated with protein expression, such as yield, are addressed in a rational and systematic way using a multiparametric approach. Optimization has been experimentally proven to increase protein expression rates up to 100-fold in a variety of host systems [4]. Figure from Fath et al. [4], used under terms of the Creative Commons Attribution License, with modification.

CRISPR-Cas9 genome editing

Invitrogen[™] TrueGuide[™] Synthetic gRNA is 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. Pair this reagent with one of our next-generation TrueCut Cas9 proteins—Invitrogen[™] TrueCut[™] Cas9 Protein v2, for most common research applications, and Invitrogen[™] TrueCut[™] HiFi Cas9 Protein, for applications that are sensitive to off-target effects—to achieve high editing efficiency in difficult targets, including targets in immune cell lines (Figure 23).

Need CRISPR-validated protocols to advance your research? Visit our Genome Editing Learning Center to access protocols and more at <u>thermofisher.com/crispr101</u>



Figure 23. Advanced Invitrogen[™] CRISPR-Cas9 genome editing tools for transfection of cancer cell lines. Genome editing of multiple gene targets was performed with TrueCut Cas9 Protein v2 and corresponding TrueGuide Synthetic gRNAs. Delivery was achieved using optimized transfection protocols and Invitrogen[™] Lipofectamine[™] CRISPRMAX[™] Cas9 Transfection Reagent in two cell lines: A549, a human lung carcinoma cell line, and MDA-MB231, a human breast cancer cell line. The graphs also compare the same experiments using products and recommended protocols from another supplier. With the Invitrogen system, cleavage efficiency is improved for low-efficiency loci (*PRKCG* and *CMPK1*) and shows consistently higher efficiency, up to a 2x increase, compared to products from the other supplier.

Clariom D Pico Assays for transcriptome analysis

Microarrays are important tools for cancer research because of their high sensitivity, robust data output, and proven track record. Applied Biosystems[™] Clariom[™] D Pico Assays provide a highly detailed view of the transcriptome with as little as 100 pg of RNA. With full coverage of the transcribed genome, including all known coding and noncoding splice variants, these microarray-based assays enable cancer researchers to detect rare and low-expressing transcripts otherwise not detected by other approaches.

On-demand webinar: Performance assessment of RNA sequencing and expression arrays for transcriptome analysis in cancer research

thermofisher.com/geneticanalysiswebinars

Petr Nazarov, PhD Research Scientist, Department of Oncology Luxembourg Institute of Health



Create physiologically relevant cell culture models

Gibco[™] Human Plasma-Like Medium (HPLM) is a new formulation designed to resemble the natural cellular environment found in the body, mimicking the metabolic profile of human plasma. HPLM contains the same salt concentrations found in human plasma, as well as the same concentrations of over 60 polar metabolites, such as amino acids, nucleic acids, sugars, and small organic acids. In resembling the natural cellular environment found in the body, HPLM provides researchers the ability to study the impact of physiologically relevant cell media on their specific applications.

Straight from the scientist

"We used HPLM with cancer cell lines from different tissues and different mutation background. Our overall impression of HPLM is quite positive. HPLM was suitable for all the tested cancer cell lines. Cells were easily and quickly adapted to the HPLM without affecting viability and cell culture performance. However, we detected relevant impact of cell proliferation, metabolism, and mitochondrial function in cells grown in HPLM as compared with classical media. After our experience using HPLM in cancer cell models, we consider it as the best choice to get more physiological data."

> –Omar Torres-Quesada, PhD, postdoctoral researcher at University of Innsbruck, Austria

To view performance data and publications, please visit thermofisher.com/gibcohplm

Microplate assays for 3D cell culture

Depending on the type of readout and level of detail needed, fluorescently labeled spheroids can be detected and analyzed with microplate readers, fluorescence microscopes, and highcontent screening systems. Selection of the optimal imaging and analysis system enables fluorescence detection throughout the entire spheroid, increasing the quality of 3D culture images and data. Poor imaging in the core of a 3D cell culture is often thought to be due to reagent penetration issues; furthermore, light scattering can cause significant limitations in spheroid imaging.

Microplate assays can be used to quickly assess cell viability and health as a preliminary measurement before downstream applications such as drug characterization. For comparative studies where analysis can be done on the medium rather than the cells (e.g., Invitrogen[™] PrestoBlue[™] HS reagent or ELISA), a microplate-based readout is the preferred method. Experience fast, accurate analysis with the Thermo Scientific[™] Varioskan[™] LUX Multimode Microplate Reader to measure fluorescence intensity with high sensitivity (Figure 24).





3D organoid and spheroid cell culture

Organoids, spheroids, and the study of cells as 3D models show great potential in many applications. These models are commonly used in cancer research because of their structural and functional similarities to *in vivo* tumors. 3D cellular models like organoids and spheroids (Figure 25) offer opportunities to better understand complex biology in a physiologically relevant context where 2D models have not proven as successful.

Gain confidence to culture and analyze organoids and spheroids through validated protocols and educational resources at <u>thermofisher.com/3dmodel</u>

Straight from the scientist

"I am really interested in how we can use organoids to model tumor pathology because so far we are only using 2D cell lines and animal models, and now thanks to the organoid technology, notably in prostate cancer, colon cancer, and pancreas cancer, we have a better input in this pathology."

-Laura Broutier, Gurdon Institute



The complex interactions between transcription, translation, and posttranslational modifications are hidden from view in typical endpoint assays that measure either RNA or protein levels. While *in situ* hybridization (ISH) and immunocytochemistry (ICC) assays are standard methods to visualize RNA and protein localization, respectively, the ability to simultaneously examine RNA and protein in a single cell has been thwarted by the incompatibility of ICC and ISH protocols. The Invitrogen[™] ViewRNA[™] Cell Plus Assay is a novel method that combines ICC with ViewRNA technology, a fluorescence ISH (FISH) and sequential branched DNA signal amplification technique, to visualize RNA and protein with single-molecule sensitivity in single cells (Figure 26).



Figure 26. Use of the ViewRNA Cell Plus Assay to examine *ERBB2* mRNA and HER2 protein expression levels simultaneously in three different human breast cancer cell lines. Using the ViewRNA Cell Plus Assay Kit (Cat. No. 88-19000-99), HER2 protein was labeled with Invitrogen[™] eBioscience[™] eFluor[™] 570 ERBB2 (HER2) antibody (Cat. No. 41-9757-82), and *ERBB2* mRNA expression was assessed with a Type 6 (Alexa Fluor[™] 647) label probe specific for *ERBB2* mRNA. *ERBB2* mRNA (white) was localized to the cytoplasm, while HER2 protein (red) was predominantly found in the membrane in both SKBR3 and BT474 cells. MCF7 cells, which do not show *ERBB2* gene amplification, were negative for *ERBB2* mRNA and HER2 protein expression. Nuclei were stained with DAPI (blue).



Figure 25. Advance your cell culture with techniques for spheroid culture and imaging. A549 cells were plated at a density of 5,000 cells/well on a Thermo Scientific[™] Nunclon[™] Sphera[™] U-bottom plate and incubated for 24 hr in a CO₂ incubator. The resulting spheroids were fixed with 4% formaldehyde and permeabilized with 0.25% Triton[™] X-100 detergent. The spheroids were then blocked with 3% BSA and stained with Ki-67 antibody conjugated to Alexa Fluor[™] 647 dye using the Invitrogen[™] Zip Alexa Fluor[™] 647 Rapid Antibody Labeling Kit. Nuclei were stained with Hoechst[™] 33342 stain. The plate was imaged with a 10x objective using confocal mode on a Thermo Scientific[™] CellInsight[™] CX7 LZR High Content Analysis Platform. The image is from a maximum-intensity projection of 20 optical Z slices of 10 µm each.

Key product listing

Empower your process with other protocols and techniques, listed here.



Cell Therapy Systems

As you move from basic cell therapy research to the clinic, high-quality products and proper

documentation are essential to getting it right the first time. Cell Therapy Systems[™] (CTS[™]) products help minimize the risk of contamination and variability in your research and provide the required documentation for regulatory review. Learn more at thermofisher.com/cts



TaqMan Liquid Biopsy Digital PCR (dPCR) Assays

Liquid biopsy is a noninvasive technique for molecular biomarker discovery that is expected to eliminate the need for costly, invasive procedures. Our wet lab-verified Applied Biosystems[™] TaqMan[™] Liquid Biopsy dPCR Assays enable the sensitive detection and quantitation of low-frequency mutations. including the most common cancer-related mutations in EGFR, BRAF, KRAS, PIK3CA, JAK2, and other genes.



QuantiGene RNA Assays

Invitrogen[™] QuantiGene[™] RNA Assays are hybridization-based assays that utilize branched DNA signal amplification technology to measure gene expression. Several short capture probes enable the detection of degraded mRNA in archival FFPE samples. QuantiGene assays work directly with cell or blood lysates or tissue homogenates without RNA purification or enzymatic manipulation. Invitrogen[™] QuantiGene[™] Plex DNA CNV Assays are also available to measure differences in copy number.



PrimeFlow RNA Assay

The Invitrogen[™] PrimeFlow[™] RNA Assay expands the capability of flow cytometry to measure RNA. It is the

only known commercial assay that enables the simultaneous detection of up to four RNA targets in combination with immunophenotyping for cell-surface and intracellular proteins using fluorophore-conjugated antibodies. The PrimeFlow RNA Assay can detect mRNA, IncRNA, and microRNA, as well as viral RNA and telomere DNA.



KingFisher Apex Purification System

The Thermo Scientific[™] KingFisher[™] Apex Purification System automates the extraction and purification of DNA, RNA, proteins, and cells. KingFisher instruments provide easy-to-follow protocols that remove manual steps and reduce overall processing time and errors associated with sample preparation while increasing the reproducibility of your yields compared to manual workflows.



Oncomine Focus Assay

The Ion Torrent[™] Oncomine[™] Focus Assay is a targeted, multibiomarker assay that enables you to target hotspots, SNVs, indels, CNVs, and gene fusions in DNA and RNA in a single workflow. Designed for clinical and translational cancer research, the Oncomine Focus Assay leverages Ion AmpliSeg[™] technology to enable robust results, low input amounts, and analysis of low-quality samples, and is compatible with FFPE fine-needle aspirates and core-needle biopsies.



Oncomine Myeloid Research Assay

Current methods for the analysis of hematological malignancies often involve multiple sequential tests and laborious workflows. The Ion Torrent[™] Oncomine[™] Myeloid Research Assay is a comprehensive, targeted NGS assay designed to assist in the understanding of myeloid cancer in a single test. It interrogates relevant DNA mutations and fusion transcripts associated with myeloid disorders in a guick and easy NGS assay.

Mata sharing

In cancer research, there is simply no room for error. Getting the most data from a single sample and ensuring the data you get are robust and reliable are critical to advancing your cancer research studies. Simple, efficient ways to share data can further accelerate productivity and enhance collaboration.

The key solutions and cloud-enabled tools highlighted in this chapter are designed to help cancer researchers achieve better productivity, improved data, and simplified data sharing.

Technology spotlight: CytoScan HD Suite, a whole-genome solution

The Applied Biosystems[™] CytoScan[™] HD Suite replaces the need to run multiple tests to obtain comprehensive cytogenetic analysis of hematological malignancy samples. This comprehensive, high-resolution whole-genome solution provides data otherwise only obtained using four different technologies. With a single CytoScan HD microarray assay you can detect chromosomal arm aberrations, focal changes, loss of heterozygosity (LOH), copy-neutral loss of heterozygosity (cnLOH), ploidy changes, chromorthripsis, and more (Figure 27).



Straight from the scientist

"The genetic complexity of cancer cells in hematological malignancies requires a comprehensive approach for detection of relevant changes. The identification of copy number gains and losses, LOH, cnLOH, clonal heterogeneity, and ploidy status as well as mosaicism are all critical for evaluating blood cancer samples to discover new biomarkers. The CytoScan HD assay has enabled us to accurately analyze many aberrations in blood cancer samples."

-Dr. Alka Chaubey, Greenwood Genetic Center

Automated or manual sample prep

- Sample types—blood or bone marrow
- Sample input-200 ng DNA
- Sample preparation—automated on the NIMBUS Target Preparation Instrument, or manual preparation



 High throughput—whole-genome copy number analysis on the Applied Biosystems[™] GeneChip[™] Scanner 3000 7G System



- Data analysis—identification of amplifications or deletions, LOH, cnLOH, ploidy, and breakpoint determination
- Integrated reporting—Oncomine Reporter for myeloid cancer

Figure 27. Streamlined workflow for more comprehensive detection and analysis of copy number changes in lymphoid and myeloid malignancies. Go from sample to answer in as little as 3 days, including analysis time.

The following technology spotlights help cancer researchers tackle data challenges frequently met during their workflow, such as data collection, integration, and analysis.

Technology spotlight: CellInsight CX7 LZR Pro High Content Analysis Platform

The Thermo Scientific[™] CellInsight[™] CX7 LZR Pro High Content Analysis Platform is a fast, laser-based, automated cell imaging and analysis platform for quantitative microscopy and phenotypic screening. It is designed to provide you with the sensitivity and speed that is needed for emerging assays (Figure 28). The CellInsight CX7 LZR Pro platform has a next-generation backside illuminated sCMOS camera with near-perfect quantum efficiency. The newly optimized Thermo Scientific[™] HCS Studio[™] 5.0 Cell Analysis Software offers intuitive interfaces and guided workflows for integrated data collection and analysis on CellInsight platforms. Additionally, the Thermo Scientific[™] ImageiT[™] Cell Painting Kit has been codeveloped with the CellInsight CX7 LZR Pro platform to enable turnkey multiparameter labeling, imaging, and analysis.



Figure 28. High-content imaging and analysis for more robust data. The CellInsight CX7 LZR Pro platform is designed for assay multiplexing using a combination of brightfield and fluorescence imaging.

NGS data analysis workflow for Ion Reporter Software



Figure 29. In five streamlined steps, you can simplify your informatics needs. (1) Automatically transfer your sequencing data from Ion Torrent[™] instruments. (2) Analyze data using preconfigured workflows optimized for a variety of applications. (3) Annotate variants from >20 public and proprietary databases, then filter for functional and biological relevance. (4) Visualize variants in Integrative Genomics Viewer (IGV). (5) Select relevant variants and create interpretative research reports, or export for further annotation in Oncomine Reporter.

Technology spotlight: Ion Reporter Software

Minimize the barriers of next-generation sequencing (NGS) data analysis and simplify your bioinformatics path to discovery. Ion Reporter[™] Software provides an optimized suite of simple tools that streamline data analysis for Ion GeneStudio[™] S5, Ion PGM[™], and Ion Proton[™] systems (Figure 29). With these tools, you can focus on finding the biological meaning of your data and spend less time on configuring and setting up software.

Easy data analysis across a variety of research applications

- Push-button data analysis with easy-to-use preconfigured and customizable workflows, providing flexibility for users of any experience level
- Easily identify germline and somatic variants with annotations from >30 public and proprietary databases, as well as cancer drivers from the Ion Torrent[™] Oncomine[™] Variant Annotator plug-in

Security for your data

- Control access with role-based logins, locked workflows, and audit logs to help maintain a traceable and secure environment
- Analyze and securely share data as part of the Thermo Fisher[™] Cloud Platform, or analyze and store data on-site with the local Ion Reporter[™] Server System

Web-based Ion Reporter Software, powered by the Thermo Fisher Cloud Platform

Ion Reporter Software as part of the Thermo Fisher Cloud Platform helps provide a convenient, centralized data analysis and management solution. Securely share your data with collaborators using the Cloud Platform who are outside of Ion Reporter Software, and easily compare data across genetic analysis techniques such as Sanger sequencing and Applied Biosystems[™] TagMan[™] real-time PCR assays, all in one place.

To access Ion Reporter Software as part of the Cloud Platform, simply log into Ion Reporter Software or log into the Cloud Platform and select the Ion Reporter Software app.

Technology spotlight: Oncomine Reporter

Ion Torrent[™] Oncomine[™] Reporter is a genomic analysis software tool developed specifically for further examination of NGS data, enabling streamlined access to a final report in just a few easy steps (Figure 30). This powerful tool is designed to help cancer researchers link variants to labels, guidelines, and clinical trials. This process of investigation helps transform data into knowledge, enabling efficiency for cancer research and future drug development.





Figure 30. Oncomine Reporter delivers a customizable report from NGS data.

Key product listing

Explore more solutions for data challenges with the following products.



Attune NxT Flow Cytometer

The unique design of the Invitrogen[™] Attune[™] NxT Flow Cytometer offers progressive application capabilities, from 1 to 4 lasers and from 4 to 14 colors, providing expanded assay development flexibility and increasing the information you can obtain from your sample. This translates into more data with better population identification than if you were using fewer parameters.



iBright Imaging Systems

Capture images and analyze data from your western blots and gels efficiently and easily using Invitrogen[™] iBright[™] Imaging Systems. These high-performance

instruments enhance the imaging experience through advanced automated features and an interface that is easy to use for researchers of all experience levels.



CytoVista 3D Cell Culture Clearing Reagent

The Invitrogen[™] CytoVista[™] 3D Cell Culture Clearing Reagent clears fluorescently labeled 3D cultured cells such as organoids and spheroids. This clearing process helps improve data quality by enabling sharp and bright 3D fluorescent imaging for depths up to 1,000 µm. The reagent can also be used as an imaging solution, with stable storage of 3D cell cultures for weeks with minimal change in tissue morphology, or brightness or specificity of the fluorophores.



OncoScan CNV Assay

See more copy number changes in your cancer research samples with the Applied Biosystems™

OncoScan[™] CNV Assay. Designed to cover the whole genome as well as known cancer-driver events, this single microarraybased assay easily detects copy number gains and losses, LOH, cnLOH, ploidy, allele-specific changes, break points, mosaicism, clonal heterogeneity, and chromothripsis.

Clariom D assays

Get more RNA expression data from a single assay and expand your potential to discover

novel, informative cancer biomarkers with Applied Biosystems™ Clariom[™] D assays. Our novel microarray-based Clariom D Pico assays provide intricate transcriptome-wide gene- and exonlevel expression profiles, including the ability to detect alternative splicing events of coding RNA and long noncoding RNA (IncRNA) in a single 3-day experiment.



Oncomine Comprehensive Assay v3

The Ion Torrent[™] Oncomine[™] Comprehensive Assay v3 is a member of the family of widely adopted Oncomine[™] assays for clinical cancer research. Oncomine assays

are NGS-based multibiomarker assays used to profile thousands of samples in different translational and clinical research projects, and have consistently delivered reliable results.



Oncomine Immune Response Research Assay

The Ion Torrent[™] Oncomine[™] Immune Response Research Assay is a targeted NGS gene expression assay that enables immunotherapy research from the quantitative evaluation of the expression of markers associated with different leukocyte subsets, antigen presentation, checkpoint pathways, and tumor progression. This 395-gene assay is supported with a user-friendly informatics workflow and demonstrates high sensitivity for low-expressing transcripts derived from FFPE samples.



Oncomine BRCA Research Assav

The Ion Torrent[™] Oncomine[™] BRCA Research Assay for the detection of BRCA somatic and germline

mutations in formalin-fixed, paraffin-embedded (FFPE) tissue and whole blood has been widely adopted in molecular laboratories across the world. The assay can detect large insertions and deletions (indels) and exon or whole-gene deletion or duplication events, uniquely empowering laboratories to detect all classes of mutations in one NGS workflow.



Oncomine Childhood Cancer Research Assay

The types of genetic aberrations associated with childhood malignancies differ from those affecting the adult population, so a specifically designed, targeted but comprehensive panel is needed to advance clinical research and drive future therapeutic solutions for precision oncology. The Ion Torrent[™] Oncomine[™] Childhood Cancer Research Assay is a unique, NGS-based tool designed for comprehensive genomic profiling of cancers affecting children and young adults.

Collaboration and community enablement

Together, we can be smarter, faster, and more determined than ever in the fight against cancer. Collaboration is the key to efficiency. We strive to bring the research community together through impactful initiatives, virtual conferences, and live events. The collaborative spirit has evolved to become stronger and even more connected. Let's continue the momentum together.

Read on for ways in which we are helping to create community and collaboration among cancer researchers.

Straight from the scientific community: antibody validation*

Experimental reproducibility is vital for the advancement of cancer research. However, the replication of studies has long been a challenge due to the lack of characterization standards for antibodies (Figure 31). Everyone from the scientific community plays a role in this challenge: suppliers, researchers, and publishers. We can increase reproducibility by collaborating in three important ways:

- Research—validate your antibody and your experimental protocol
- **Publish**—use and list your controls and standards along with details about antibodies and experimental methods
- **Train**—expand your knowledge about experimental methods and controls, sample preparation, and other aspects of properly using antibodies



Figure 31. Ongoing state of the antibody selection challenge. Data from Baker M (2015) [3].



Thermo Fisher Scientific has implemented methods for the verification and reporting of antibody specificity to further enhance data transparency. These specificity tests help ensure that the antibody will bind to the correct target.

See "Purchase with confidence" on **page 16** to learn more about how performance-tested antibodies help save money.



Advanced Verification

You can easily identify antibodies that have already undergone this additional testing with the Advanced Verification badge. This badge can be found in antibody search results and on appropriate antibody product webpages. The additional data supporting the Advanced Verification status can be found in the product-specific data image galleries.

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

Giving researchers a platform: Gibco Cell Culture Heroes

Gibco[™] Cell Culture Heroes spotlights PhD students and postdoctoral scientists across the globe with the primary focus of telling the story of their cancer research. This program promotes education and creates a growing community of researchers who are driving tomorrow's breakthroughs in the fight against cancer. Do you want to be highlighted as one of our next Gibco Cell Culture Heroes? Submit an application at thermofisher.com/cellcultureheroes

Hear from current Gibco Cell Culture Heroes

Visit <u>Twitter.com</u> and search #CellCultureHeroes.



"So honored to be one of the first @GIBCO @thermofisher #CellCultureHeroes! I have just received this stupendous trophy! Truly amazed. Thank you."

-Kristine Wadosky, PhD



"Thank you @GIBCO for this truly unique trophy! I'm proud to be a part of the #CellCultureHeroes team! DMEM will be joining my cancer and *E. coli* cell family."

-Leah Persaud, PhD candidate



"Exciting parcel in the mail today. An amazing trophy for #CellCultureHeroes from @GIBCO. It's been such a great experience being involved and I love the trophy!"

-Sophie Cowman, PhD candidate



A virtual event: 5 Days of Stem Cells

Our annual Gibco[™] 5 Days of Stem Cells virtual event takes place every fall. Registration is free and the event can be accessed from anywhere around the world. Here is what cancer researchers can expect:

- Scientific presentations—over 30 prominent researchers and thought leaders in the industry, live or on-demand, sharing their research breakthroughs and insights
- Education center—access virtual training labs, handbooks, career development resources, and more
- Expanded training opportunities—receive live behind-thescenes training with our stem cell experts, and connect with our live stem cell support for tips and tricks
- **Networking**—expand your research network and enrich your perspective through the lens of our global audience

Register for this year's event at thermofisher.com/5daysofstemcells

User spotlight: mass spectrometry

Community empowerment for mass spectrometry users comes in the form of the Thermo Scientific[™] Tandem Mass Tag[™] (TMT[™]) Research Award. For research users of Thermo Scientific[™] TMT[™] labeling reagents (including amine-, sulfhydryl-, or carbonyl-reactive reagents), you could receive one of two awards that provide up to \$11,000 worth of these reagents, free for use in your doctoral or postdoctoral research.* Apply for a TMT Research Award at **thermofisher.com/tmtgrant**

Straight from the scientist

"The tandem mass tag reagents have spawned a multiplexing revolution in quantitative proteomics, transforming the mass spectrometer into a robust experimental platform for investigating cellular systems. In my laboratory, we are using TMT [reagents] to define the molecular mechanisms of tumor metastasis."

-Noah Dephoure, assistant professor at Weill Cornell Medical College, TMT Research Award recipient

Want to start saving money today? Take advantage of the Thermo Scientific[™] Mass Spec Rewards Program and get a 40% discount quote on a single order, valid globally for researchers and core facilities. Learn more at thermofisher.com/msrewards

* Terms and conditions apply. For complete details, go to thermofisher.com/tmtgrant

Additional resources

Education and enrichment

Cancer Research Hub

We have consolidated in one place all of the technologies and resources we offer to help you at every stage of your research journey—and will continue to maintain and update this vital source. Let a new era of collaboration in the fight against cancer begin here and now.

thermofisher.com/cancerresearch

Immuno-oncology Research Hub

Immuno-oncology is considered by some to be one of the most promising areas of research in the world of anticancer therapeutics. Aimed at generating a cancer immunotherapy, immuno-oncology manipulates the body's own immune response to mount an attack against tumor cells. Visit this hub as a onestop resource.

thermofisher.com/immuno-oncology

Gibco Cancer Basics

Explore Gibco[™] Cancer Basics, an introduction to cancer cell culture that covers topics such as the biology of cancer, cell line culture, and the culture of cancer spheroids. Whether you are new to cancer cell culture or are looking to enhance your knowledge, Gibco Cancer Basics helps you culture your career. thermofisher.com/cancerbasics

Protein and cell analysis eLearning courses

Get free access to e-courses and educational materials about topics and techniques relevant to cancer research, such as antibody validation, flow cytometry, protein gel chemistries, western detection, mass spectrometry, ELISAs, immunoassays, imaging, and more.

thermofisher.com/proteincelledu

School of Molecular Biology

The School of Molecular Biology is here for you, whether you want to review the basics, gain more in-depth knowledge, or discover our latest research tools. This is an educational hub for molecular biology, with rich and reliable technical content designed for new and experienced molecular biologists alike. Tap into our free educational resources to advance your cancer research.

thermofisher.com/molbioschool

Oligos, primers, probes, and genes

This portal provides a one-stop hub for custom genomics products for cancer researchers. Order custom-synthesized oligos, primers, probes, and genes for PCR, cloning, hybridization, real-time PCR, protein expression, and more. Each service includes easy ordering tools and convenient delivery times.

thermofisher.com/oligotools

Synthetic Biology Learning Center

Synthetic biology combines molecular biology and systems biology with engineering principles to design biological systems and biofactories. The aim is to create improved biological functions to address current and future challenges, including the fight against cancer. Learn about available custom services as well, from cloning and expression to host engineering. thermofisher.com/synbiolearningcenter

Scientist to Scientist events

The Scientist to Scientist (S2S) Program features technical and educational content presented worldwide by the research scientists who developed the technologies. Check out the interactive map at the following link to find S2S events occurring in your area. You can also contact your Thermo Fisher Scientific account manager to request an event.

thermofisher.com/s2s

Field application scientist services

If you have questions related to protein quantitation techniques or any immunoassay product or instrument, we offer valuable in-person consultation services. A highly trained field application scientist can visit your lab or assist you or your team by phone in any of the following ways, free of charge. Services include:

- Protein quantitation education
- Training on the benefits of multiplexing
- Technical questions answered
- Help getting started with your immunoassay
- Hands-on immunoassay training
- Immunoassay troubleshooting thermofisher.com/fascontact

Real-time PCR education

Whether you're new to real-time PCR, also called quantitative PCR (qPCR), or want to learn about new applications for real-time PCR, we have learning materials, including videos and webinars, to help you understand the technology and get started quickly. thermofisher.com/qpcreducation

Technical support

Global services and support

Find answers online, training services, and more. Cancer researchers have direct access to more than 1,400 experienced technical support scientists worldwide. In addition, our application-specific support centers contain a wealth of useful resources including frequently asked questions, getting started tips, and troubleshooting help.

thermofisher.com/support

Compliance, validation, and calibration services

To help meet requirements and mitigate risk in both regulated and nonregulated environments, Thermo Fisher Scientific offers a broad array of audit compliance, quality compliance, and validation services, ranging from risk assessment and hardware or software qualification to full system validation. thermofisher.com/complianceservices



Service plans Planned maintenance and quick response times to help you avoid unnecessary downtime, reduce strain on laboratory staff, and extend the life of your instruments



Compliance services Timely, cost-effective, and audit-ready documentation managed by a compliance specialist to help ensure your instrument is installed, operating, and performing to the manufacturer's specifications



Analytical validation (AV) consulting services Technical project management, data analysis support, and documentation of your lab's AV are provided to help you develop and optimize your workflow for required parameters



Bioinformatics and IT services Optional consulting services with a bioinformatics applica

bioinformatics application scientist to review software, applications, workflow optimization, and data management



Education services Application and instrument training programs are available at our training centers located throughout the world, within your lab, or through web-based instruction

Ordering information

Ordering information

Product	Quantity	Cat. No.	
Time savers			
Attune Flow Cytometers	thermofisher.com/attune		
Bigfoot Spectral Cell Sorter	thermofisher.com/b	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	
CellTrace CFSE Cell Proliferation Kit	1 kit	C34554	
LIVE/DEAD Fixable Dead Cell Stain Sampler Kit	1 kit	L34960	
Mini Gel Tank	1 unit	A25977	
iBlot 2 Gel Transfer Device	1 device	IB21001	
iBind Western Device	1 device	SLF1000	
iBright Imaging System	1 unit	FL1000	
Instant ELISA kits	thermofisher.com/findelisa		
Microplate readers and washers	thermofisher.com/platereaders		
E-Gel Power Snap Electrophoresis Device	1 device	G8100	
Platinum II Taq Hot-Start DNA Polymerase	100 reactions	14966001	
KingFisher Apex Purification System	1 unit	5400920	
CTS Dynabeads CD3/CD28	10 mL	40203D	
Oncomine Pan-Cancer Cell-Free Assay	8 reactions	A37664	
Purchase with confidence			
MAPK signaling pathway antibodies	Find antibodies for cancer research at thermofisher.com/antibodies		
AKT signaling pathway antibodies			
Phosphospecific antibodies			
Transcription-specific antibodies			
GTPase antibodies			
Alexa Fluor secondary antibodies			
Alexa Fluor Plus secondary antibodies			
HRP secondary antibodies			
Qubit Flex Quantitation Starter Kit	1 kit	Q45894	
ProQuantum immunoassay kits	thermofisher.com/proquantum		
Gibco media and Nunc plastics	thermofisher.com/bettertogether		
Uncoated ELISA kits	thermofisher.com/findelisa		
MiniAmp Thermal Cycler	1 each	A37834	

Ordering information

Product	Quantity	Cat. No.
Efficiencies for precious samples		
Ion GeneStudio S5 System	1 each	A38194
Qubit 4 Quantitation Starter Kit	1 kit	Q33239
MagMAX Cell-Free DNA Isolation Kit	1 kit	A29319
MagMAX Cell-Free Total Nucleic Acid Isolation Kit	1 kit	A36716
MagMAX FFPE DNA/RNA Ultra Kit	1 kit	A31881
SuperScript IV VILO Master Mix	50 reactions	11756050
Oncomine TCR Beta-LR Assay	24 samples	A35386
Oncomine TCR Beta-SR Assay, DNA	1 kit	A39072
Oncomine TCR Beta-SR Assay, RNA	1 kit	A39359
Oncomine Tumor Mutation Load Assay, Chef-ready library preparation	32 samples	A37910
Arcturus Cellect LCM System	thermofisher.com/arcturus	
Clariom S Pico Assay, human	30 assays	902929
Clariom D Pico Assay, human	30 assays	902925
TaqMan Mutation Detection Assay	75 reactions	4465804
TaqMan Advanced miRNA Assay	250 reactions	A25576
SuperScript IV Reverse Transcriptase	2,000 units	18090010
Platinum SuperFi DNA Polymerase	100 units	12351010
PureLink RNA Micro Scale Kit	50 preps	12183016
SYBR Gold Nucleic Acid Gel Stain	500 μL	S11494
SuperSignal West Femto Maximum Sensitivity Substrate	20 mL	34094
Bolt 4–12% Bis-Tris Plus Gels, 10-well	1 box	NW04120BOX
Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	1 mL	A1413201
Advanced techniques made accessible		
OpTmizer T Cell Expansion Serum-Free Medium (SFM)	1 L	A1048501
TaqMan Liquid Biopsy Digital PCR (dPCR) Assays	450 reactions	A44177
	40 assays	88-18005-204
PrimeFlow KINA Assay Kit	100 assays	88-18005-210
Oncomine Focus Assay	48 samples	A29230
Oncomine Myeloid Research Assay	32 reactions	A36941

* 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.

Ordering information

Product	Quantity	Cat. No.
Improved data and data sharing		
CytoScan HD Suite	24 assays	901835
CellInsight CX7 LZR Pro High Content Analysis Platform	thermofisher.com/hcs	
Ion Reporter Software	1 each	4487118
Ion GeneStudio S5 System	1 unit	A38194
Oncomine Reporter	1 each	A34298
CytoVista 3D Cell Culture Clearing Reagent	30 mL	V11315
OncoScan CNV Assay	24 assays	902695
Oncomine Immune Response Research Assay	32 samples	A32881
Oncomine Myeloid Research Assay	24 reactions	A36940
Oncomine Childhood Cancer Research Assay	24 reactions	A36485
Collaboration and community enablement		
Antibody Validation* Inititative	thermofisher.com/antibodyvalidation*	
Gibco Cell Culture Heroes	thermofisher.com/cellcultureheroes	
Gibco 5 Days of Stem Cells	thermofisher.com/5daysofstemcells	
Thermo Scientific Tandem Mass Tag (TMT) Research Award	thermofisher.com/tmtgrant	

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References

- The National Cancer Institute. What Is Cancer? Available at: cancer.gov/about-cancer/ understanding/what-is-cancer (accessed October 2018).
- The National Cancer Institute. Cancer Statistics. Available at: cancer.gov/about-cancer/ understanding/statistics (accessed October 2018).
- 3. Baker M (2015) Reproducibility crisis: Blame it on the antibodies. Nature 521:274-276.
- Fath S, Bauer AP, Liss M et al. (2011) Multiparameter RNA and codon optimization: a standardized tool to assess and enhance autologous mammalian gene expression. *PLoS One* 6(3):e17596.
- Bradbury A, Pluckthun A (2015) Reproducibility: Standardize antibodies used in research. Nature 518:27–29.

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