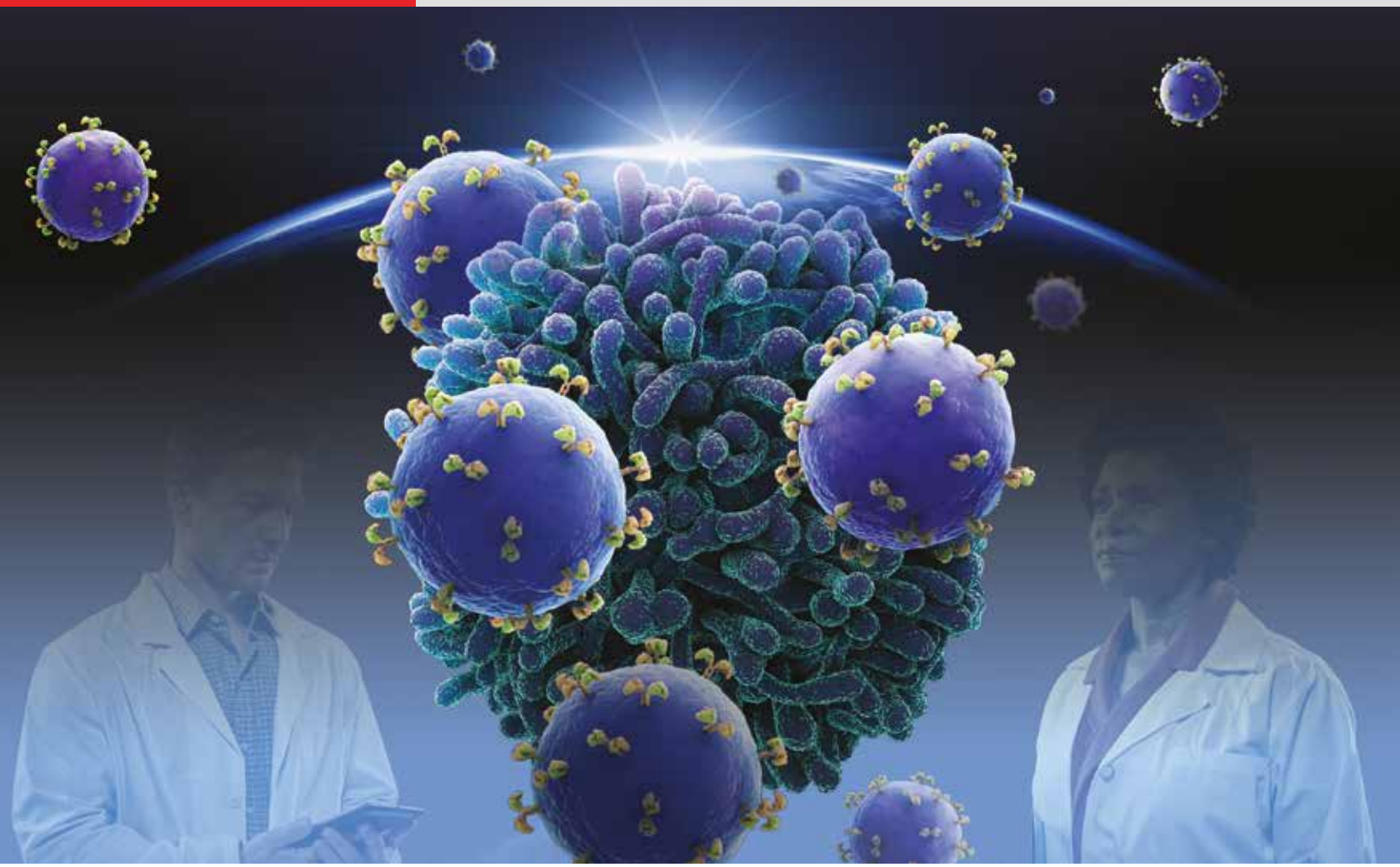
life IN THE lab

PRODUCTS, INFORMATION, AND SCIENTAINMENT

ISSUE 24 | AUTUMN/WINTER 2018

Exploring Disease Immunity



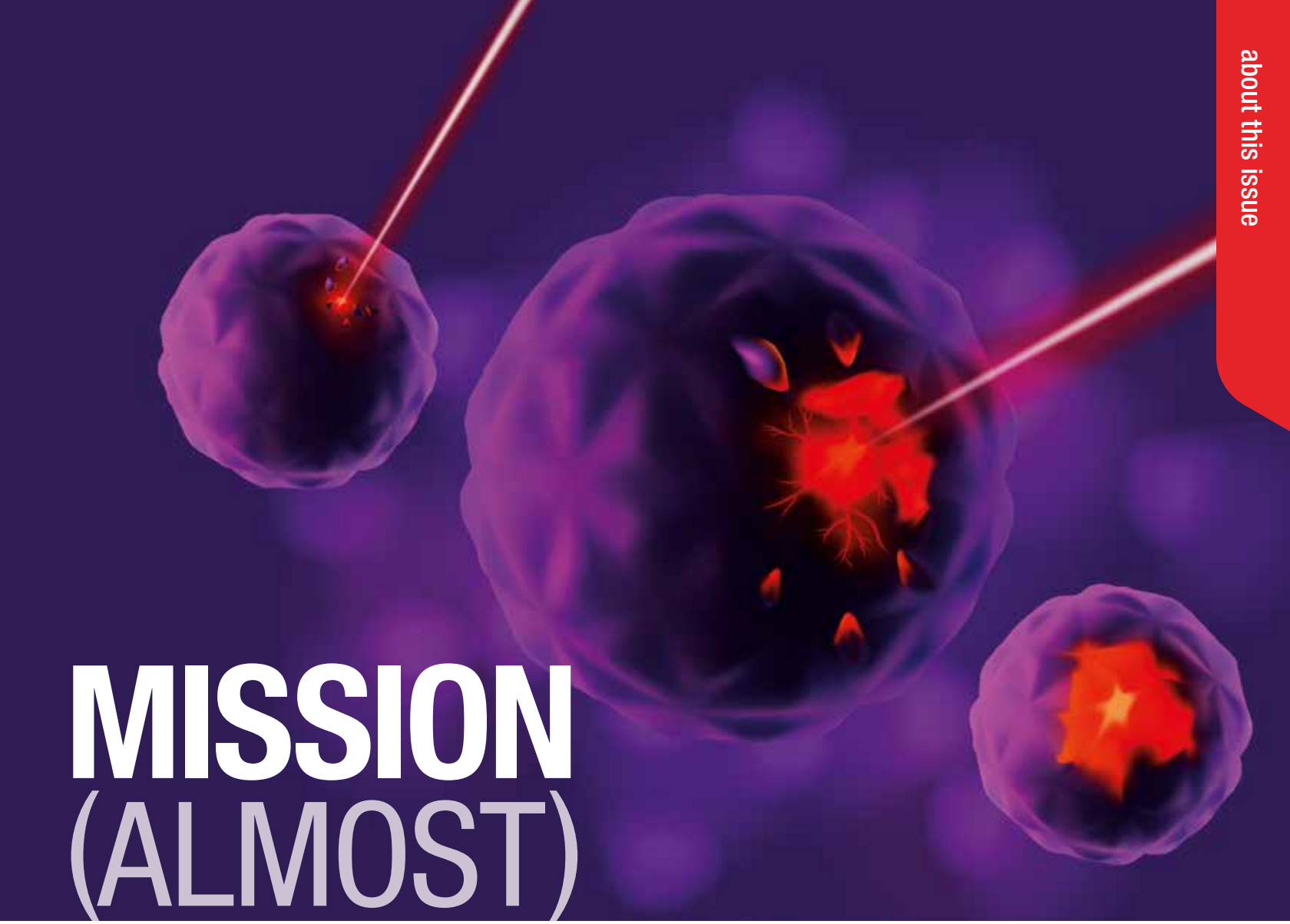
Venture past the verge

You're closer than you may think to making meaningful advances in immuno-oncology

Immuno-oncology has the potential to significantly change the way we think about and treat cancer. We have the innovative technologies, educational tools, and expertise to help you go from discovery, through validation, to creating cancer immunotherapies.

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

ThermoFisher SCIENTIFIC



MISSION (ALMOST) ACCOMPLISHED

Researchers in immunology fight challenges old and new

Investigation of disease states has taken researchers on a complex journey of uncovering the inner workings of the immune system and its responses to pathogens. Throughout this issue we explore both immunology and a variety of diseases, highlighting how continued innovation can improve treatments.

But did you know that some deadly diseases are still lurking around, although they were thought to have been almost completely eradicated? Recent examples include:

- **Bubonic plague**—over 200 deaths in Madagascar were recorded in 2017
- **Scarlet fever**—it mysteriously resurfaced in England and East Asia
- **Syphilis**—the 90s had the highest rate of infection in 40 years
- **Polio**—while 99% eradicated, it still exists in Afghanistan, Nigeria, and Pakistan
- **Leprosy**—treatable, but it takes three to five years for symptoms to show, making eradication difficult
- **Cholera**—1 million cases in Yemen in 2017, the largest outbreak in recent history

These sobering statistics illustrate the need for continued research and vigilance when it comes to diseases and the immune system.

invitrogen

INVESTIGATING INFLAMMATION

Choosing the right antibodies to understand the complex process of inflammation

Inflammation is the body's response to tissue damage. The accompanying pain, warmth, and swelling are all hallmarks of normal biological processes that remove pathogens in the body. Immune cells swarm to the affected area and release cytokines and other factors to remove damaged tissue and start healing. Even nonpathogenic conditions like touching a hot stove can start the inflammation cascade. Limited inflammation is protective and necessary to keep the body running effectively.

But, what happens when inflammation is not well regulated? This type of inflammation can be found in chronic conditions. A disease like atherosclerosis is accompanied by the production and release of inflammatory factors.[1] Plaques on vessel walls have immune cells and platelets producing molecules that regulate adhesion and lipid uptake. Atherosclerosis can be controlled by diet, activity, and medication; and yet, there is a need to understand the underlying dysregulation of the inflammation process.[2,3] Greater knowledge of inflammation could lead to better ways to control these types of diseases.

NEW PANEL BUILDER FOR FLOW CYTOMETRY

Dissecting the complexity behind inflammation in chronic diseases requires analysis of markers in immune cells, cytokines, or other proteins.[4,5] The Invitrogen™ Flow Cytometry Panel Builder makes panel design simpler, helping you design your panel more efficiently regardless of your level of experience. With this tool, you can analyze your markers of interest and get closer to your next discovery.

Learn more at **thermofisher.com/order/panel-builder**

1. Voloshyna I, Littlefield MJ, Reiss AB (2014) Atherosclerosis and interferon-γ: New insights and therapeutic targets. *Trends Cardiovasc Med* 24:45-51.
2. Libby P (2012) Inflammation in atherosclerosis. *Arter Thromb Vasc Biol* 32:2045-2051.
3. Xue-Qiao Z (2018) Pathogenesis of atherosclerosis. [online] Available at: uptodate.com/contents/pathogenesis-of-atherosclerosis
4. Graves AJ, Padilla MG, Hokey DA (2014) OMIP-022: Comprehensive assessment of antigen-specific human T-cell functionality and memory. *Cytometry A* 85:576-579.
5. Cossarizza A and Cousin D (2015) Overcoming challenges in cellular analysis. *Science* 347: 443 [online] Available at: sciencemag.org/custom-publishing/webinars/overcoming-challenges-cellular-analysis-multiparameter-analysis-rare?_ga=2.88382104.2078857589.1534352862-1479773893.1534352862

BOOST YOUR IMMUNITY WITH THESE TIPS

When it comes to maintaining a healthy immune system, every little bit helps. Below are a few of our favorite methods.

DREAM IN THE DARK

And we mean total darkness, because our circadian rhythm is programmed for two 12-hour periods of light and darkness. By eliminating light pollution (yes, even from cell phones), you're giving your body better rest and recovery. What's more, research has linked the genes responsible for our internal clock to immune cells.[1] So draw the curtains and nix the night light.

LAUGH IT OFF

A good guffaw or series of chuckles has been found to actually decrease hormones associated with stress.[6] Regulating your mood with laughter increases endorphins, which have a positive effect on psychological health as well as the immune system. And that's definitely no joke.

MUNCH ON MICRONUTRIENTS

It's best to naturally ingest micronutrients in your regular meals, but turning to dietary supplements is a popular alternative. Deficiencies in vitamin B6, vitamin C, vitamin D, vitamin E, magnesium, and zinc have been linked to immune dysfunction,[4] so make sure you don't skip out on your vitamins.

FIND YOUR CENTER WITH MEDITATION

Over a quarter of the global population is affected by anxiety and depression. In a 2017 study, meditation helped regulate the stress response, suppressing chronic inflammation and helping to maintain a healthy gut microbiome.[5] Meditation may sound daunting at first, but even five minutes daily can help you de-stress and come back to center. Find a quiet place, get yourself into a comfortable position and just focus on breathing.

VINYASA FLOW YOUR WAY INTO BLISS

Chronic stress is damaging to the immune system over time.[2] It's possible that the balance between the sympathetic and parasympathetic nervous systems can be positively impacted by yoga. In a study with 60 students, those practicing yoga experienced fewer autonomic and psychologic changes when compared to a control group.[2] In yet another study, yoga resulted in an increased circulation of immune-related cytokines, which pointed toward a healthier immune system.[3]

1. *Science* 342:727 (2013).
2. *Int J Yoga* 41:26 (2011).
3. *J Altern Complement Med* 21:530 (2015).
4. *Immunol Cell Biol* 94:117 (2016).
5. *Adv Mind Body Med* 3:10 (2017).
6. *J Exp Med* 239:243 (2016).

CELLULAR IMMUNOLOGY

Payal Damani-Yokota, PhD
University of Massachusetts, Amherst
Cellular and Molecular Immunology
@PayalYokota

The scope of her research and the questions she is asking:

For the last 6 years, I have been working toward my PhD at the University of Massachusetts in Amherst under the tutelage of Dr. Cynthia L. Baldwin. The overarching goal of our research is to uncover the signals that drive development and function of gamma delta (γδ) T cells. What makes these cells especially interesting is that they express a family of scavenger receptor cysteine-rich (SRCR) glycoproteins called Workshop Cluster 1 (or WC1) that functions as a hybrid pattern recognition receptor/co-receptor exclusively in conjunction to the γδ TCR. Our research previously showed that WC1-expressing γδ T cells are indispensable for response to bacterial antigens such as *Leptospira*; and upon engagement with ligand, the endodomains can signal via phosphorylation of conserved serines and tyrosines. We have also found that in response to *Leptospira*, one subset of WC1$^+$ cells produces IFNγ, while the other does not. While we knew a lot about WC1 function and its importance in recall responses, we did not know how many WC1 molecules a single γδ TCR$^+$ cell could express, or how many of these molecules were required to direct immune responses by γδ T cells. It was also not known when and where these molecules are first detected during T cell development, or how these functional subsets of γδ T cells were transcriptionally programmed.

Some answers:

To answer these questions, I flow-sorted purified populations of WC1$^+$ γδ T cell subsets and developed a culture system such that I could expand 78 different WC1$^+$ γδ T cell clones from a single cell origin where primary γδ T cells could proliferate *in vitro* for up to 10 weeks. Using [Applied Biosystems™] TaqMan® Assays, I evaluated WC1 gene transcription on each clone to evaluate the WC1 gene expression. The co-transcription of WC1 genes showed that WC1s can be expressed by themselves or in combinations with some overlap between the two subsets. Despite this overlap, the *Leptospira*-responsive WC1$^+$ memory γδ T cell clones showed significantly higher propensity to express WC1 molecules that are known to bind to the pathogen. I also found that WC1 molecules are exclusively expressed on thymic γδ T cells and stratify into the mostly nonoverlapping subsets early in development. This is particularly important because unlike conventional αβ T cells, γδ T cells bifurcate into functional subsets during thymic development guided by distinct transcriptional programming.

The next steps:

My insatiable curiosity about where and how γδ T cells develop and why and how they function has led me to investigate even more. The beauty of innate-like lymphocytes is that they have the clonotypic TCRs as well as accessory receptors that help them to respond early and fast, and to expand despite being tiny populations in blood. γδ T cells act as bridges between the innate and adaptive immunity, thereby making for an elegant, synchronous, pathogen-combating immune system. On a global note, my research has led me to ask if and where such lateral systems can exist in humans and mice, where PRR/co-receptor molecules partake in recognition of complex antigens and can help in producing functional responses to protect against "damaged/stressed self" as well as "non-self." So, I am packing up the lessons learned from my PhD and taking them "to go," as I embark on a venture toward cancer immunology in my next gig as a postdoc.

ACHIEVE OVER 90% TRANSFECTION AND EDITING EFFICIENCY IN IMMUNE CELLS

Delivering molecules into blood cells is challenging. At the same time, genetic manipulation of blood cells is necessary for developing treatments for a broad range of diseases such as leukemia, solid tumor cancers, and HIV infection. With our new application note and step-by-step protocol, you can achieve over 90% transfection efficiency immune cells and over 90% editing efficiency in primary T cells.

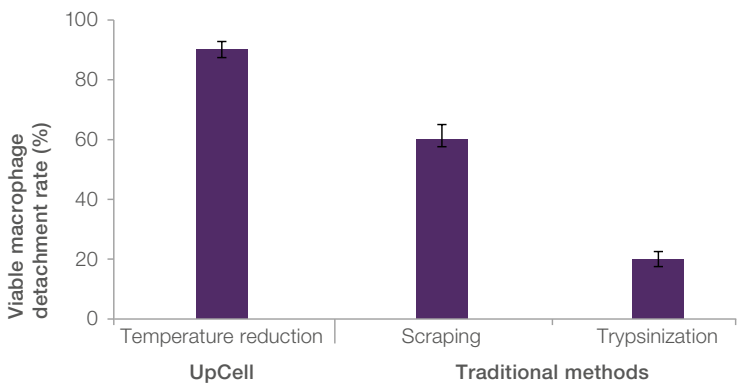
Find the application note and more at **thermofisher.com/neon**

A CULTURE SURFACE THAT ENABLES CELL DETACHMENT BY TEMPERATURE REDUCTION

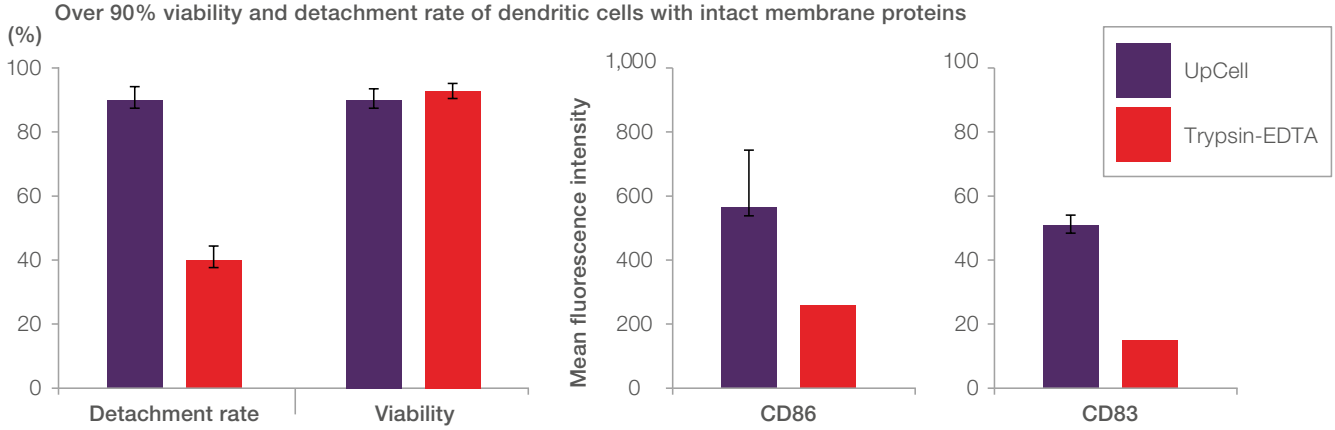
Enable non-enzymatic harvesting of adherent cells for the preservation of cell viability and surface proteins. The temperature-responsive surface of Thermo Scientific™ Nunc™ UpCell™ dishes revolutionizes how you prepare your cells for cellular analysis and functional assays.

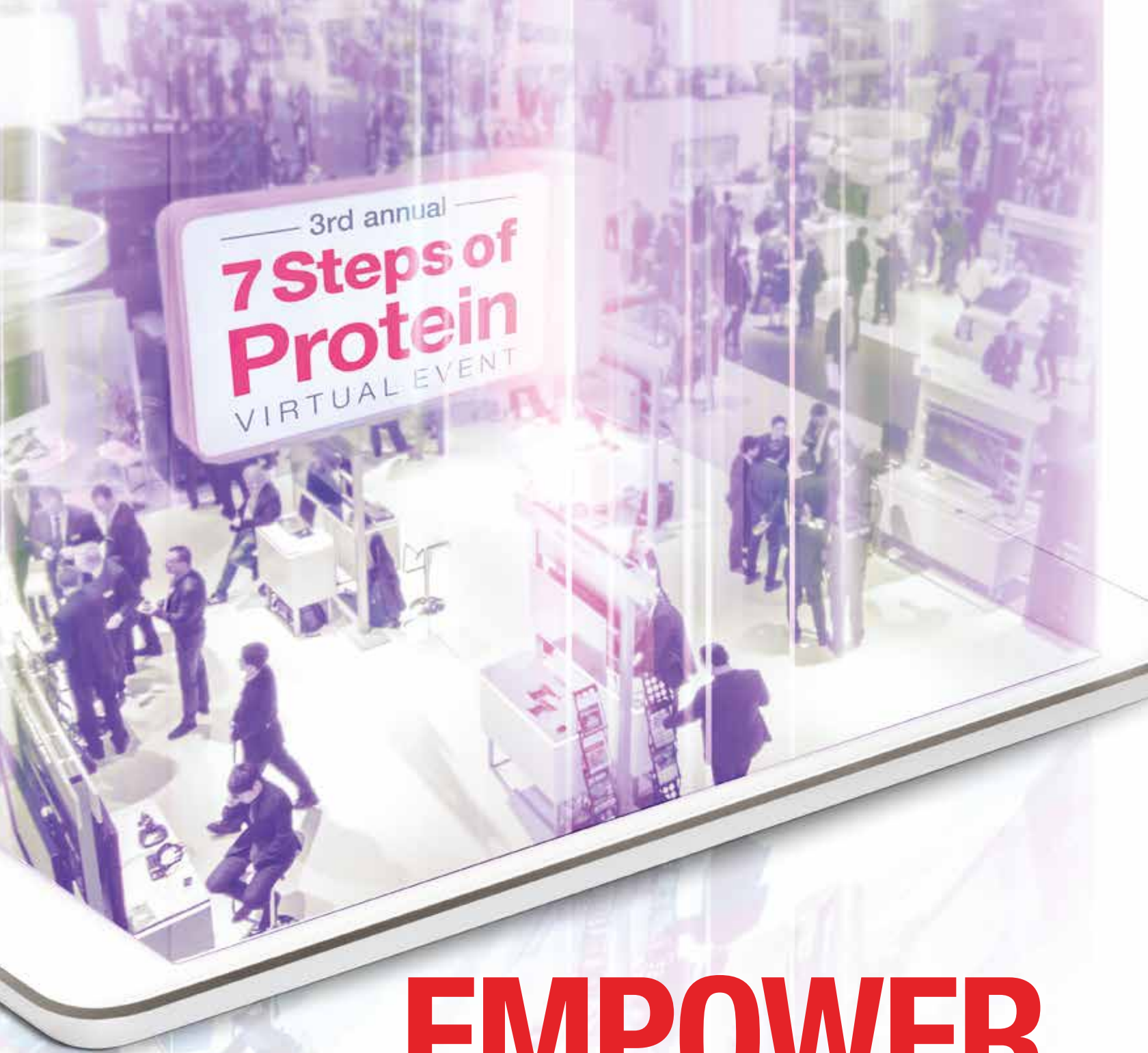
thermofisher.com/upcell

Over 90% viable macrophage detachment on UpCell dishes



Over 90% viability and detachment rate of dendritic cells with intact membrane proteins





EMPOWER
YOUR PROTEIN RESEARCH

Each and every step of the way

REGISTER FOR ACCESS NOW

The 3rd annual 7 Steps of Protein Virtual Event is now available **on demand**.

This virtual event offers both broad and in-depth content, designed to give you the information and insights you need to empower your protein research every step of the way. You'll receive firsthand knowledge of the latest technologies, as well as tips and tricks for common protein research areas and applications.

During the 7 Steps of Protein event you can:

- Attend valuable webinars and poster presentations—by scientists, for scientists—to learn from industry experts how to optimize your protein biology results
- Chat live with speakers and get your questions answered in real time
- Access a wide selection of resources, such as technical handbooks, white papers, how-to-use product videos, and product selection guides
- Receive free Continuing Education (CE) credits for the presentations you attend

thermofisher.com/7steps

Step 1: Protein expression

Step 2: Protein isolation & purification

Step 3: Gel electrophoresis

Step 4: Western blotting

Step 5: Protein assays & analysis

Step 6: Mass spectrometry

Step 7: Crosslinking & modification

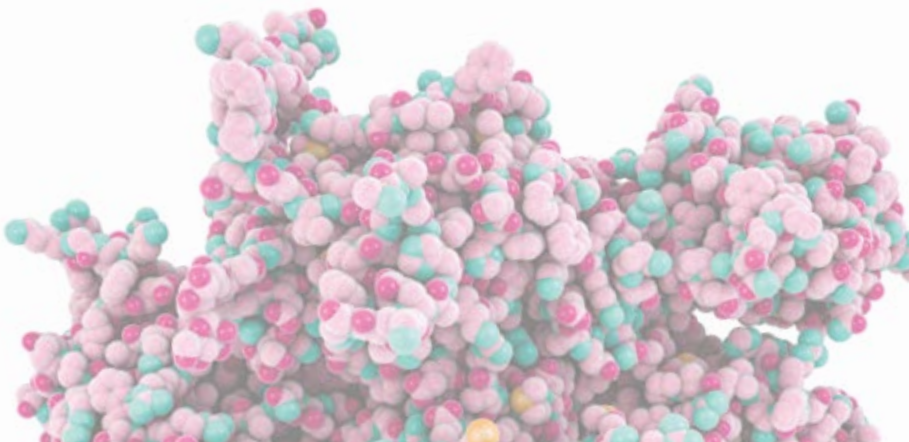
SPEAKERS

Paul Haney, PhD
Senior Product Manager, Protein and Cell Analysis, Thermo Fisher Scientific
Webinar: Light Up Your Western Blots—Fluorescent Western Blotting Tips, Tricks, and More

Voula Kodoyianni, PhD
Product Marketing Manager—Materials and Structural Analysis, Thermo Fisher Scientific
Webinar: Protein Sample Evaluation Using the Thermo Scientific™ NanoDrop™ One UV-Vis Spectrophotometer

David Bourdon, PhD
Senior R&D Manager, Antibodies and Immunoassays, Thermo Fisher Scientific
Webinar: Invitrogen™ ProQuantum™ High-Sensitivity Immunoassays Offer Minimum Sample Consumption with Maximum Performance

Other presentations and webinars cover topics ranging from detection of multiple autoimmune diseases to analysis of signaling pathways like AKT/mTOR. To see the full list of presentations and webinars, go to **thermofisher.com/7steps**



READY, SET, AUTOMATE!

HIGH-THROUGHPUT AND AUTOMATED SOLUTIONS TO ADVANCE YOUR RESEARCH

A common challenge with research in immunology and disease is the vast diversity found in immunogens, antibodies, and other associated immune-response molecules. You need to count on technology and products that can handle large numbers of samples and a large multiplex of analytes. When compounded with the number of clinical research samples to be processed, a high-throughput setup becomes even more critical. Our automated platforms, instruments, and reagents for molecular biology enable you to examine more data than ever before, providing the speed and confidence you need to find a therapeutic solution faster.

Thermo Fisher Scientific offers everything you need to automate your workflow, from nucleic acid isolation systems to incubators and plate movers.

thermofisher.com/labautomation

DESIGNED FOR EASY ROBOTIC INTEGRATION

The Applied Biosystems™ Automated Thermal Cycler can be run on or off a robotic platform. Validate your assay using our intuitive software, and then integrate the Automated Thermal Cycler when you're ready.

Flexible service plans for your Automated Thermal Cycler help minimize downtime and help reduce repair costs.

thermofisher.com/atc

SUPERIOR STABILITY OF PCR ENZYMES AT THE BENCH

PCR enzyme stability is key to a successful automated workflow setup. Invitrogen™ DNA polymerases containing Platinum™ hot-start technology allow for benchtop stability at room temperature for at least 24 hours.

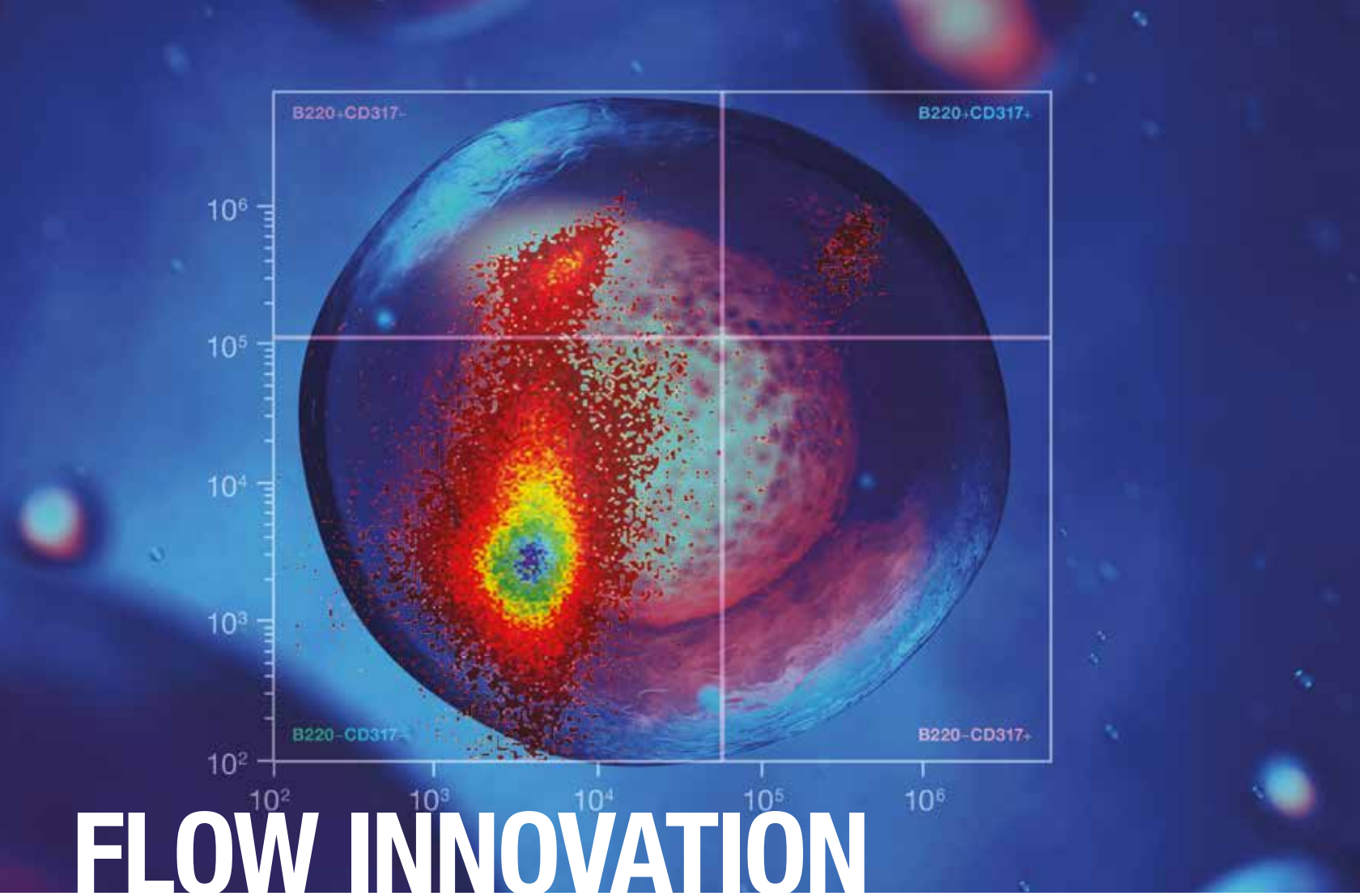
- Platinum™ II *Taq* Hot-Start DNA Polymerase enables universal primer annealing at 60°C, with 4x faster cycling times
- Platinum™ SuperFi™ DNA Polymerase helps ensure sequence accuracy with >100x *Taq* fidelity

thermofisher.com/platinumiitaq
thermofisher.com/platinumsuperfi

FLEXIBLE TRANSFORMATION OPTIONS TO MATCH YOUR AUTOMATED THROUGHPUT NEEDS

The ability to perform transformations both manually and on an automated liquid handler is critical to optimizing your throughput. Using Invitrogen™ MultiShot™ competent cells, you can choose from 1 to 96 transformations based on your specific application.

thermofisher.com/multishot



FLOW INNOVATION ADVANCES HIV RESEARCH

One researcher's pursuit of rare T cell analysis using flow cytometry

Sara De Biasi, PhD
Department of Life Sciences
University of Modena and Reggio Emilia (UNIMORE)
Modena, Italy

Immunotherapies transform the body's inherent immune system, elevating its ability to fight cancer. T cells and their mediated responses are central to this transformation. In acquired immunodeficiency conditions such as HIV, T cells are fatally targeted by the virus in a sophisticated effort to hinder the body's immune system. Scientists like Sara De Biasi are achieving immense insights on the activities and characterization of T cells, specifically invariant natural killer T cells (iNKT) in HIV-positive subjects. Using flow cytometry technology to inch closer to uncovering revolutionary immunotherapies, Sara shares her research journey—one driven by natural curiosity and an early exposure to the mesmerizing interplay between HIV and the immune system.

What drives your passion for science?
I am a very curious person. In every field, I aspire to know how and why something happens—what are the causes, what are the consequences. The more complex it is, the more passionate I become.

What inspired you to pursue this profession?
My childhood dream was to become a medical doctor and perform autopsies. However, I decided to start my studies by

gaining a solid background in biology. Thus, I took a degree program in biotechnology. I was so enthusiastic to understand the molecular and cellular mechanisms responsible for an immunological disorder that I realized I had found my way—to this day, it's still the most appealing aspect of my research.

How did you first become involved in researching acquired immunodeficiencies, such as HIV?
Professor Andrea Cossarizza was my professor of immunology. His enthusiasm was contagious, and I was extremely curious to understand how the immune system works during HIV infection and how HIV kills $CD4^+$ T cells. He was looking for students to carry on HIV research; in particular, the effects and side effects of antiretroviral therapy. I applied and started the day after.

You're interested in rare populations of T cells and their role in autoimmune diseases. Can you share more?
I have good training and experience in immunology, particularly in the development and use of new flow cytometric approaches. My longstanding research commitments are centered on identifying the molecular and cellular basis and involvement of the immune system in several diseases and infections, such as HIV/AIDS or hepatitis and physiopathological conditions. I have built expertise in the clinical application of new methods for the identification of rare cellular subsets in patients affected by HIV infection and in patients undergoing liver transplantation, as well as in patients suffering from multiple sclerosis. Such methods are allowing a new characterization of the functional activities of these cells.

What challenges do you face in your research?
Every time I am dealing with a new project, there are at least two big things to think about: how to choose the right methodology and how to deal with huge amounts of data. Communication is crucial to achieving knowledge and results, so it's paramount to feel comfortable reaching out to colleagues for help. Moreover, I work at the university, so one of my activities is to teach students.

With multiparameter capabilities and a very high analysis rate, flow cytometry is, at present, the most potent technology to address rare cell analysis.

I explain how to work in the lab and emphasize that productivity is the most valuable quality a researcher should possess. Teaching has been a challenging endeavor; but in the end, I realize that I have empowered potential new researchers and I'm proud of that.

In your research journey, you've leveraged flow cytometry and continue to be an advocate for this technology. Can you share more?
Flow cytometry is fast and precise, and it allows the study of several dozens of proteins expressed at a single cell level. The study of rare cell populations is of growing importance. It's useful not only to understand disease mechanisms, but also to find novel targets. 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 HIV, as HIV-positive people are immunocompromised). More than 20 million cells should be stained to find these rare populations among PBMCs. Until recently, no instrument was able to acquire 20 million events. Then the Invitrogen™ Attune™ NxT Flow Cytometer made my project reliable. The consequence of acquiring 20 million cells was how data should be managed, but powerful computers with many gigabytes solved this problem.

What is ISAC and what is it like being one of their scholars?
The International Society for Advancement of Cytometry (ISAC) aims to advance the impact of cytometry in the sciences. ISAC has a worldwide membership of more than 1,850 scientists. I became a scholar in 2016 and can network with top experts, strengthen my critical thinking and independent approach to science, and develop a solid foundation for my career. Thanks to the scholarship, I obtained the Italian National Scientific Habilitation to become Associate Professor.

How will the work you've done impact the research community at large? What's the potential of applying flow cytometry technology to other areas of research?
The most recent immunotherapies use an approach that reshapes the body's own immune system to fight dangerous cancer cells that may have disguised themselves or put the brakes on the immune system. The recent immuno-oncology therapies are revolutionizing treatment in some patients with certain types of cancers. In tumors such as melanoma, the results have been even more impressive, but many patients unfortunately will develop progressive disease. Thus, it is important to understand immune checkpoint inhibitors and the molecular mechanisms that lead to the discovery of biomarkers that are likely to predict an individual's response to therapy.

As for last thoughts to leave us with—what do your potential findings mean in the long term, for those affected by HIV?
HIV patients with a relatively advanced age, e.g., more than 50 years old, can experience pathologies that affect much older non-HIV citizens. Chronic inflammation and immune activation, observed typically in elderly people and defined as "inflammaging," can be present in HIV patients, who experience a type of premature aging. This relatively new condition is extremely complex. It is important to understand the role of inflammation and immune activation in HIV patients in order to design strategies that can support the most potent approaches.

gibco

EDUCATE YOURSELF

GIBCO™ EXPRESSIONWORLD™ VIRTUAL EVENT

Experience the latest advances in protein expression in one virtual event.

The world's largest online conference unveiling cutting-edge technology for protein expression is now available on demand. This interactive forum connects experts from pharma, biotech, and academia to share case studies and best practices in the exciting areas of biologics and vaccine development from discovery to production.

Stream now at **thermofisher.com/expressionworld**

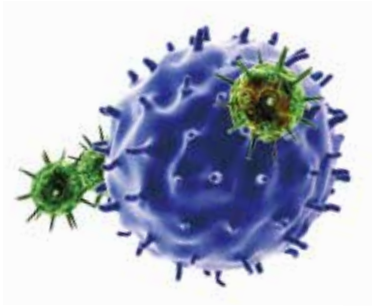
STAY ON THE LEADING EDGE OF STEM CELL RESEARCH

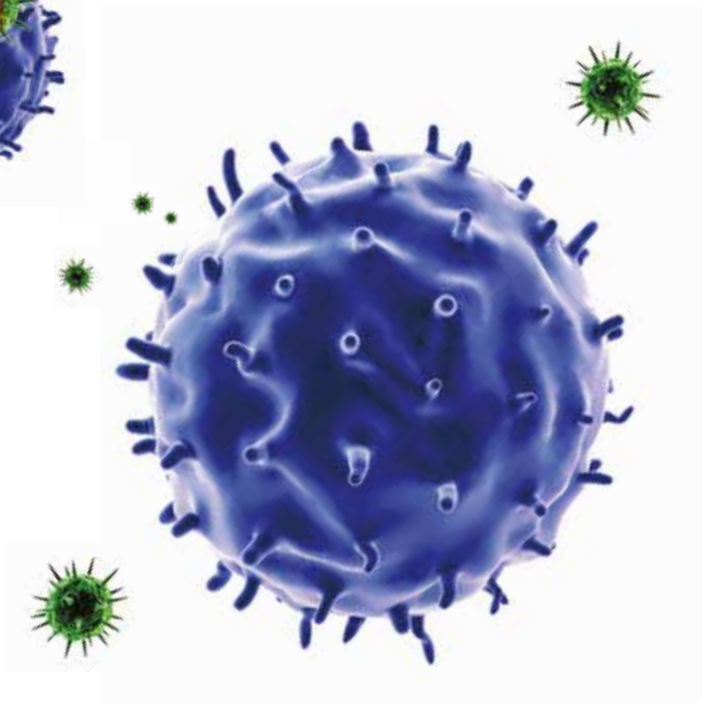
Join us for the 6th annual Gibco™ 24 Hours of Stem Cells™ virtual event, where you can:

- Access leading scientific presentations from thought leaders around the world
- Receive real-time training, tips, and technical support from our stem cell experts
- Sharpen your stem cell skills and expertise with virtual training and certifications
- Expand your research network and enrich your perspective in a global context

It's all happening live on **November 15, 2018**.

thermofisher.com/24hours





4 STEPS TO ACTIVATE T CELLS

RETAIN *IN VIVO*–LIKE FUNCTION, WITHOUT FEEDER CELLS

Adoptive cell transfer (ACT) is a rapidly emerging immunotherapy approach in which a patient's own genetically engineered immune cells are used to attack and treat their cancers. The most promising type of ACT comes from chimeric antigen receptors (CARs), where a patient's T cells are harvested and scientists add a receptor that binds to a protein on the cancerous cells. This technique is possible thanks to extensive research performed on activation and expansion of T cells.

Here are four easy steps to activate T cells without using feeder cells, but with results similar to *in vivo* activated cells.

1. Count T cells to control the ratio of beads to cells. **Pro tip:** Counting cells is an important step to maintain reproducibility.

2. Isolate T cells from mononuclear cells or whole blood cells using a negative isolation method like the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit. **Pro tip:** Your cells are not exposed to the stress of being passed through a column when you use a negative isolation method.

3. Transfer cells to desired cell culture medium. Add CD3/CD28 beads and mix gently. **Pro tip:** No need for antigen-presenting cells (APCs), mitogens, antigens or feeder cells. This technology outperforms traditional home-brew methods for generic activation (mitogens, ConA, soluble antibodies, etc.) and is well documented in the published literature.

4. Transfer to cell culture plate and incubate at 37°C in a cell culture incubator. **Pro tip:** Sit back and relax as your T cells get activated.

thermofisher.com/activate

CRISPR IS AN IMPORTANT TOOL FOR T CELL MODIFICATIONS

Several T cell–related therapies are already in the clinic, including checkpoint inhibitors and CAR T cells. Since it allows scientists to precisely edit the genome, CRISPR is a valuable tool for T cell research. With CRISPR technology, T cells can be altered to improve their ability to destroy cancer cells. For best results, start with pure populations of T cells. Invitrogen™ Dynabeads™ bead-based kits highly enrich T cell populations without adding contaminants.

thermofisher.com/dynabeads

invitrogen

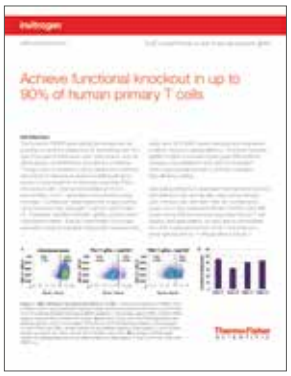
IMPACTING IMMUNITY AT THE GENETIC LEVEL

New ways to unlock the secrets of T cells

T cells are an important part of the adaptive immune system, and highly diverse T cell receptors (TCRs) allow the T cell to specifically recognize a wide range of antigens. Therefore, it is important to study TCRs in order to learn more about T cell diversity and specificity.

Sequencing and analyzing TCR genes, combined with the ability to specifically edit TCRs with CRISPR technology, will enable future T cell research. See how our synthetic biology solutions, from Invitrogen™ GeneArt™ Gene Synthesis to CRISPR-Cas9 genome editing products, are making a real difference with scientists who are trying to push the boundaries of working with T cells.

ACHIEVE UP TO 90% KNOCKOUT



CRISPR gene editing in primary T cells

T cells are emerging as important targets for cancer immunotherapy research. Obtaining high gene editing efficiency with CRISPR technology can be challenging, especially in primary cells. Compounding this challenge, primary cells are precious, and often difficult to obtain and expand. A new application note describes a complete solution for gene editing in primary T cells from cell isolation to verification of edit. This includes T cell isolation and activation using Dynabeads bead-based kits to gene editing using Invitrogen™ TrueCut™ Cas9 Protein V2, TrueGuide™ Modified Synthetic sgRNA, and the Invitrogen™ Neon™ Transfection System.

Combining Cas9 protein with sgRNA for high editing efficiency

TrueCut Cas9 Protein V2 and TrueGuide Modified Synthetic sgRNA have significant advantages over plasmid-based systems. CRISPR plasmids remain in the cell for up to 72 hours posttransfection compared to less than 24 hours with protein. This leads to fewer off-target effects or instances of nonspecific cutting. Using this method allows researchers to bypass transcription and translation steps, speeding up the process.

thermofisher.com/tcellknockout

Interested in a CRISPR training course? Go from design to analysis in 4 days.
thermofisher.com/crisprworkshop

PARTNERSHIP FOR PROMISING SOLUTIONS

Peter Ebert, PhD
Scientist
Adaptive Biotechnologies

Tell us about your company; what goals you are trying to achieve?
We are, at heart, an immune receptor sequencing company. We do T cell and B cell receptor sequencing at very high throughput and we use the precision of the adaptive immune system to develop therapy for patients. More recently, we have a tie-up with the Microsoft™ Healthcare NExT initiative to combine high-throughput immunosequencing with artificial intelligence, to map the entire human immune system. This “antigen mapping” would allow for better diagnosis; for example, if you have a particular sequence in large numbers in your T cell repertoire, it might be an indication that you can have a reaction against a particular antigen, which could be valuable for diagnostic purposes.

How does GeneArt Gene Synthesis help with your research?
We do immune receptor sequencing at very high throughput and discover millions of sequences at a time, and the biggest challenge was validating these sequences at a pace that would not be a hold-up. We found a great partner in the GeneArt Gene Synthesis team, which gave us the ability to have those constructs made efficiently. We just make a list of sequences, hand it over to them, and then in about three weeks we get the final product. In addition, we also use the GeneArt subcloning services, as we need to shuttle these sequences between series of vectors depending on the function. We then test the sequences in different systems, so that the sequences can eventually be transferred to the cells of interest. We have now evolved our bottleneck and therefore, having this convenient partner is really valuable.

What do you expect this partnership to bring in the near future?
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.

invitrogen

FIVE TIPS

Custom HRP Secondary | Antigen | Primary | Alexa Fluor Secondary | Primary Conjugated

FOR FINDING YOUR RIGHT ANTIBODY

Antibodies are used in a wide variety of research areas, and their performance and consistency have a direct effect on your results. Set yourself up for success by using these five tips to streamline your antibody selection.

Explore over 85,000 antibodies at **thermofisher.com/antibodies**

Search antibodies on the Fisher Scientific website at: **eu.fishersci.com/go/antibodysearch**

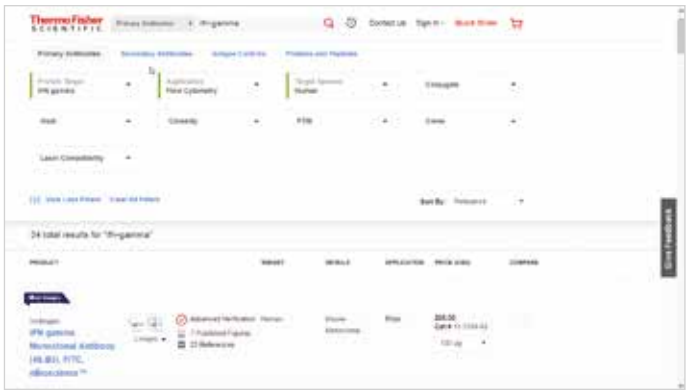
* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

TIP 1 BOOKMARK THE ANTIBODY SEARCH ENGINE

Enter your target of interest and narrow your results by application and target species based on your experimental needs.

TIP 2 REFINE SEARCH RESULTS WITH FILTERS

Modify and narrow results by antibody host species, clonality, posttranslational modification specificity, and other filters.

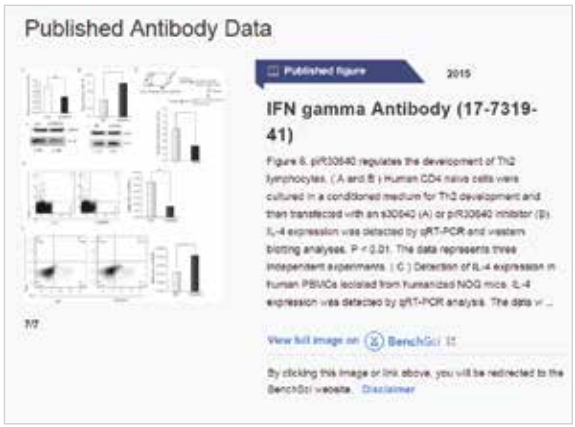


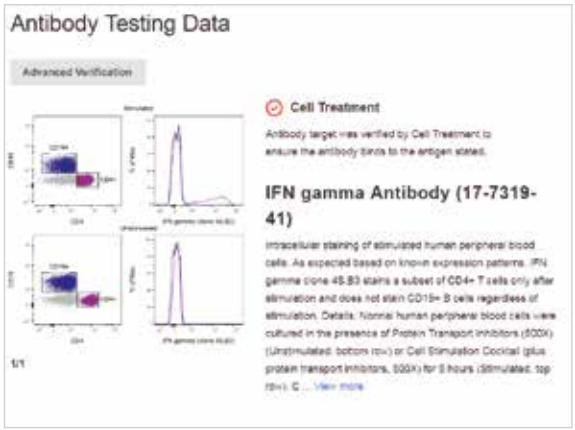
TIP 3 CONSIDER ANTIBODIES THAT HAVE BEEN VALIDATED*

The Advanced Verification icon indicates that the antibody has been tested for both target specificity and for use with a particular application.

TIP 4 REVIEW ANTIBODY APPLICATION DATA FROM MANY SOURCES

Our published antibody data page, through a partnership with the BenchSci™ platform, provides over 50,000 images showing application figures from peer-reviewed journals, along with our own extensive antibody testing data.





TIP 5 VERIFY YOUR ANTIBODY PERFORMANCE IN PUBLISHED ARTICLES

References, organized by application, provide links to citations in scientific journals.

FLUORESCENT DETECTION IN 3D CELL MODELS

Simple tools for studying complex biology in a physiologically relevant context

Traditional two-dimensional (2D) cell culture models lack many of the environmental conditions that cells experience within an organism. Their physical and biochemical setting is drastically different. For this reason, researchers have been turning to cell models grown in three dimensions (3D). These are commonly referred to as either organoid or spheroid systems. Organoids and spheroids show great potential in many applications, including drug discovery, toxicology, and disease modeling. These 3D cell models offer opportunities to better understand complex biology in a physiologically relevant context.

Cells grown in 3D typically exist in a more biochemically relevant state and differ in morphology and function, but growing them is a large investment in time and resources.

We provide analysis tools that researchers already use with confidence in 2D culture. These tools can now be easily applied to the 3D model system to advance discovery at a faster pace.

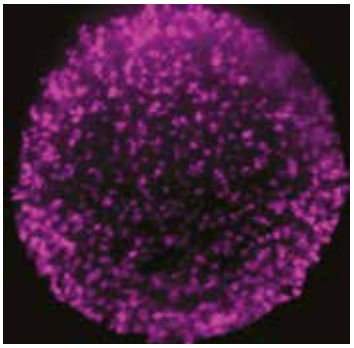


Figure 1. HeLa spheroid cells were stained with Ki67 antibody labeled with Alexa Fluor 647 (using the Zip Alexa Fluor 647 rapid labeling kit). Cells were imaged on a Thermo Scientific™ CellInsight™ CX7 LZR high-content analysis (HCA) instrument using confocal mode.

ANTIBODY DETECTION

Immunofluorescence is an essential technique for the study of any disease state. It is typically done using a primary antibody to detect the desired protein within cells and a labeled fluorescent secondary antibody to provide detection via a fluorescent signal. Three-dimensional models are more dense than 2D models. They present new challenges to labeling and may need careful optimization. Common problems are increased background fluorescence and labeling heterogeneity. A solution to these issues is to label primary antibodies directly. Direct labeling reduces the complexity of optimization; multiple primary antibodies can be used together without problems of cross-reactivity, and nonspecific background issues are often reduced.

Your favorite primary antibody markers can be easily and rapidly labeled with Invitrogen™ Zip Alexa Fluor™ Antibody Labeling Kits (Figure 1).

VIABILITY, PROLIFERATION, AND HEALTH

Functionally, organoids and spheroids differ from 2D models because there are zones with varied levels of oxygen, nutrients, and metabolites. Apoptotic or necrotic regions are typically observed at the core, while zones of proliferative cells can be detected along the periphery. Simple live/dead assays can be performed to monitor the formation and health of organoids and spheroids (Figure 2).

Mitochondria play a central role in cell life and cell death. An increasing number of studies place mitochondrial dysfunction at the heart of disease. It is essential to be able to monitor mitochondrial health in the context of cell death in model systems (Figure 3).

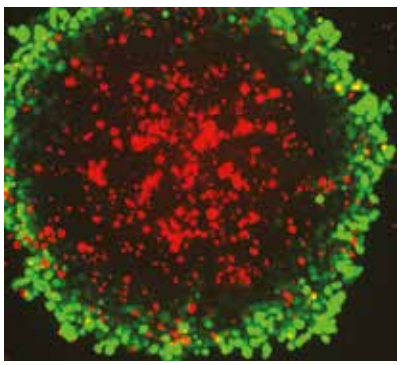


Figure 2. Confocal image of live spheroids of Alexa Fluor 594 cells stained with the Invitrogen™ LIVE/DEAD™ Cell Imaging Kit. Imaged on the CellInsight CX7 LZR HCA platform. Areas of dead cells are typically observed at the center of the 3D culture.

Confirming that 3D cell structures are maintaining the appropriate physiological morphology is paramount to reaching successful research outcomes. Our powerful imaging and high-content analysis platforms combined with our reagents are ideal for analyzing organoid and spheroid cultures (Figure 4).

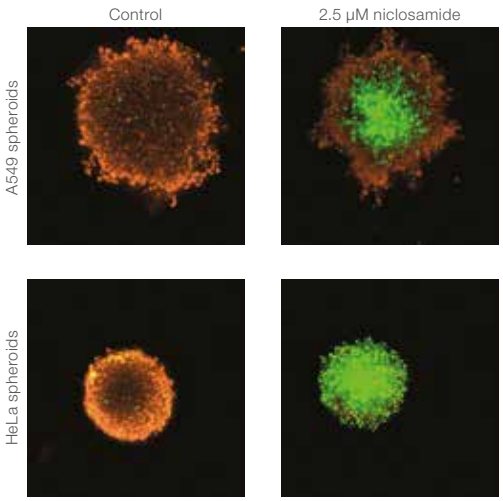


Figure 3. Monitoring mitochondrial health and apoptosis in spheroids. Spheroids were treated with different doses of niclosamide for 24 hrs, stained with Invitrogen™ MitoTracker™ Orange dye and Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent, and imaged with the CellInsight CX7 LZR HCA instrument. Increasing doses of niclosamide lead to depolarization of mitochondria (orange) and increase in apoptosis (green).

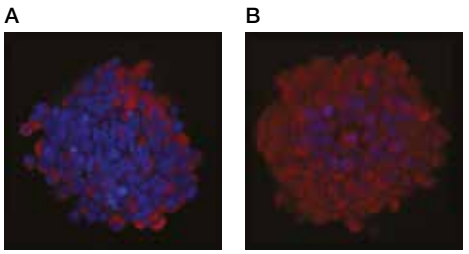


Figure 4. Spheroid staining using Invitrogen™ CellROX™ Deep Red Reagent. HeLa spheroids were **(A)** left untreated or **(B)** pretreated with 10 µM menadione. Cells showing oxidative stress are stained red and live-cell nuclei are stained blue.

Kingfisher instruments: Automate your DNA, RNA, and Protein prep

- Benchtop instruments for low to ultra-high throughput (or) Benchtop instruments for 6-96 samples
- Kits and Reagents from trusted brands to enable multiple applications
- Dedicated specialists to support your KingFisher
- Specialized plastics, protocol software, and interchangeable magnetic heads for optimized results

Learn more **thermofisher.com/kingfisher**

ThermoFisher SCIENTIFIC

BEHIND THE LAB COAT:

MEET IMMUNOLOGY RESEARCHER CASSANDRA LYBECK

Cassandra Lybeck, Master of Science in Biochemistry candidate at Ottawa Hospital Research Institute

How did you end up here? Why did you become a scientist? What drew you to this field?

Growing up in a small town in rural Saskatchewan, I didn't really know what a scientist was, so it wasn't always what I pictured myself doing. Every year in grade school we attended the annual science fair. One year my partner and I came up with a research-based project looking into what the difference was between gas and diesel engines. We ended up making it to regionals and winning a Bright Minds award. This was probably the first experience that drove me in this direction.

Choosing a major in university was a challenge because I'm interested in almost everything—so rather than jumping right in after high school, I joined the military as a signal operator. My favorite subject was radio theory, and I really enjoyed creating improvised antennae to propagate different radio frequencies. I knew at this point that I was going to be a scientist, but I was not sure what field I wanted to study.

What study path did you take to get into this field?

My path was not very typical; I changed my mind many times. After coming out of the military, I enrolled in geology. It seemed like a great choice at first, but I didn't find it very interesting and did horribly. It was very discouraging, until I started taking biology and chemistry classes. Eventually, I transferred into biomedical sciences and this led into graduate studies in biochemistry. Now I study a specific adenovirus protein, and how it is regulated during infection.

What is a typical day like in your lab?

Our lab studies the biology of adenoviruses, as well as adenovirus-based vectors for gene therapy. We explore oncolytic applications, as well as investigating potential treatments for neuromuscular dystrophies. To study how adenovirus replicates in cells, we infect them and use a variety of different molecular tools to measure its growth (or lack thereof). We create adenovirus-based vectors by removing the genes that are responsible for efficient replication, and we use different cellular or animal models (depending on the application) to look at the therapeutic impact.

Do you have a memorable breakthrough moment or favorite memory in the lab?

At the start of my project, I had to create and optimize a protocol that would allow us to use affinity purification to isolate an adenovirus protein that contained an epitope tag. The whole process revolved around infecting cells, lysing them in such a way to preserve protein–protein interactions, and then determining how much bait protein was needed to visualize this on a silver stain. Using this protocol, we were able to purify this adenovirus protein and identify interacting partners using mass spectrometry. Once we identified novel targets, I was able to look into epigenetic regulation, and this is what I'm working on now.

Why is your research important? What are the possible real-world applications?

Studying basic science in a hospital setting has its challenges, primarily because it can be hard to see the clinical relevance. I study a very specific adenovirus protein and what is happening to it during late infection times: is it being modified? Are there important novel interactions taking place? Looking at the epigenetics can tell us how this protein is being regulated and what that means in terms of virus replication. Adenovirus is common and ubiquitous in nature; it's found worldwide and infects almost every vertebrate. Since it usually results in the common cold, it doesn't pose a threat to most healthy individuals; but certain serotypes can cause debilitating illness and mortality, especially in individuals whose immune system is compromised or developing. Understanding how the virus works gives us a better insight into how to treat it. In addition, we can potentially take this knowledge and apply it to other pathogens that may behave in the same way.

You obviously enjoyed science right from the beginning, but you must have met challenges along the way. Do you have any advice for junior scientists facing challenges?

I think it's common for young scientists to doubt what they have accomplished, especially if they compare themselves with others. Imposter syndrome is such a real thing, and something that I used to struggle with (and sometimes still do). My advice would be that we are all good enough and worth it. Give yourself a goal and try to follow through. You can only do your best, so if you are having a hard time with your project or a difficult experiment is bringing you down—don't let it discourage you. Failure is an important part of learning, but it doesn't mean that we are inadequate. Just remember, we are all trying to make sense of this amazing, complex, beautiful world we live in.

WE'RE HERE TO HELP YOU SAVE

30% OFF BENCHTOP INSTRUMENTS AND **25% OFF** ASSOCIATED REAGENTS, ACCESSORIES AND CONSUMABLES*

Promotion expires 30th November 2018.

SAVE UP TO 30%

See representative or visit the fisher scientific website to find out more and to order.

Cell Engineering products: **eu.fishersci.com/go/benchtoppromo-cellengineering**

DNA and RNA Detection products **eu.fishersci.com/go/benchtoppromo-dna-rna**

Protein Analysis products: **eu.fishersci.com/go/benchtoppromo-proteinanalysis**

Lab Essentials: **eu.fishersci.com/go/benchtoppromo-labessentials**

*Terms and conditions for this promotion: Offer valid in Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Luxembourg, The Netherlands, Norway, Poland, Spain, Sweden, Switzerland and United Kingdom only unless otherwise stated for orders placed between 3rd September and 30th November 2018 in connection with the products listed above. Orders must include the relevant promotional codes, as listed on thermofisher.com/benchtopsavings. Subject to country and dealer participation. No redemption necessary. Valid while supplies last. All pricing information and values based on current list price. All offers subject to Thermo Fisher standard terms and conditions of sale. Not valid in connection with any other offer or discount. Void where prohibited by law or company policy. Please note that the value of the specific offer(s) the customer may receive from the manufacturer under this program can be considered as a gift or an advantage, and as a result pursuant to applicable local law, it could give rise to declaration, notification, publication or reporting. The customer also warrants it will comply with any local law relating to transparency, as the case may be.

Life in the Lab, Issue 24, October 2018 • "Life in the Lab" is printed by Thermo Fisher Scientific • 102 Fountain Crescent, Inchinnan Business Park, Paisley, PA4 9RF thermofisher.com/lifeinthelab • For Research Use Only. Not for use in diagnostic procedures, unless otherwise indicated.

For more information, contact Fisher Scientific

Austria: +43 (0)800 20 88 40 **Belgium:** +32 (0)056 260 260 **Denmark:** +45 70 27 99 20
Finland: +358 (0)9 8027 6280 **France:** +33 (0)3 88 67 14 14 **Germany:** +49 2304 932-5
Netherlands: +31 (0)20 487 70 00 **Norway:** +47 22 95 59 59 **Spain:** +34 902 239 303
Sweden: +46 31 352 32 00 **Switzerland:** +41 (0)56 618 41 11 **UK:** +44 (0)1509 555 500

fisher scientific

For Research Use Only. Not for use in diagnostic procedures.