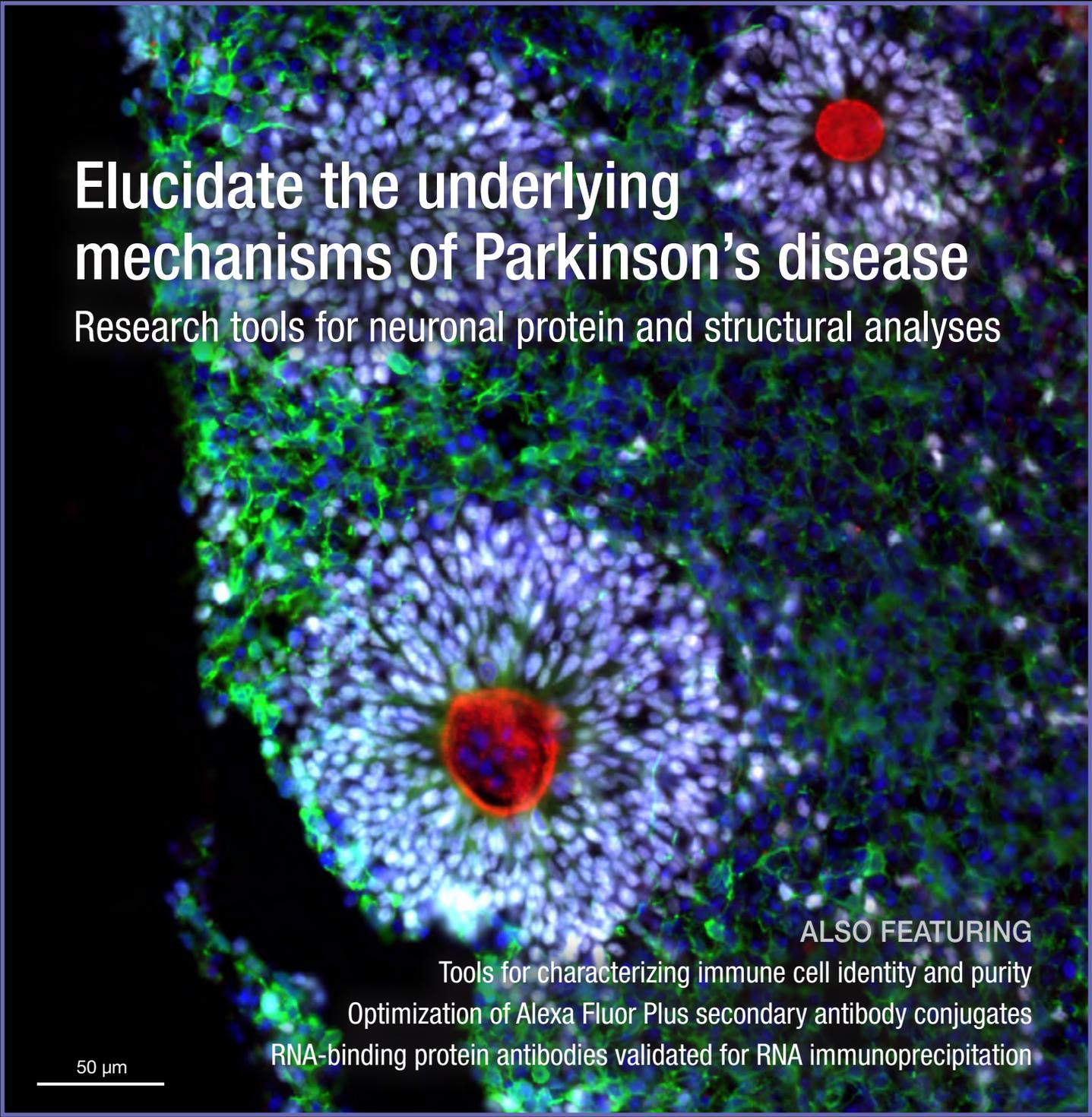


BIOPROBES 80

JOURNAL OF CELL BIOLOGY APPLICATIONS

WINTER 2019



Elucidate the underlying mechanisms of Parkinson's disease

Research tools for neuronal protein and structural analyses

ALSO FEATURING

Tools for characterizing immune cell identity and purity

Optimization of Alexa Fluor Plus secondary antibody conjugates

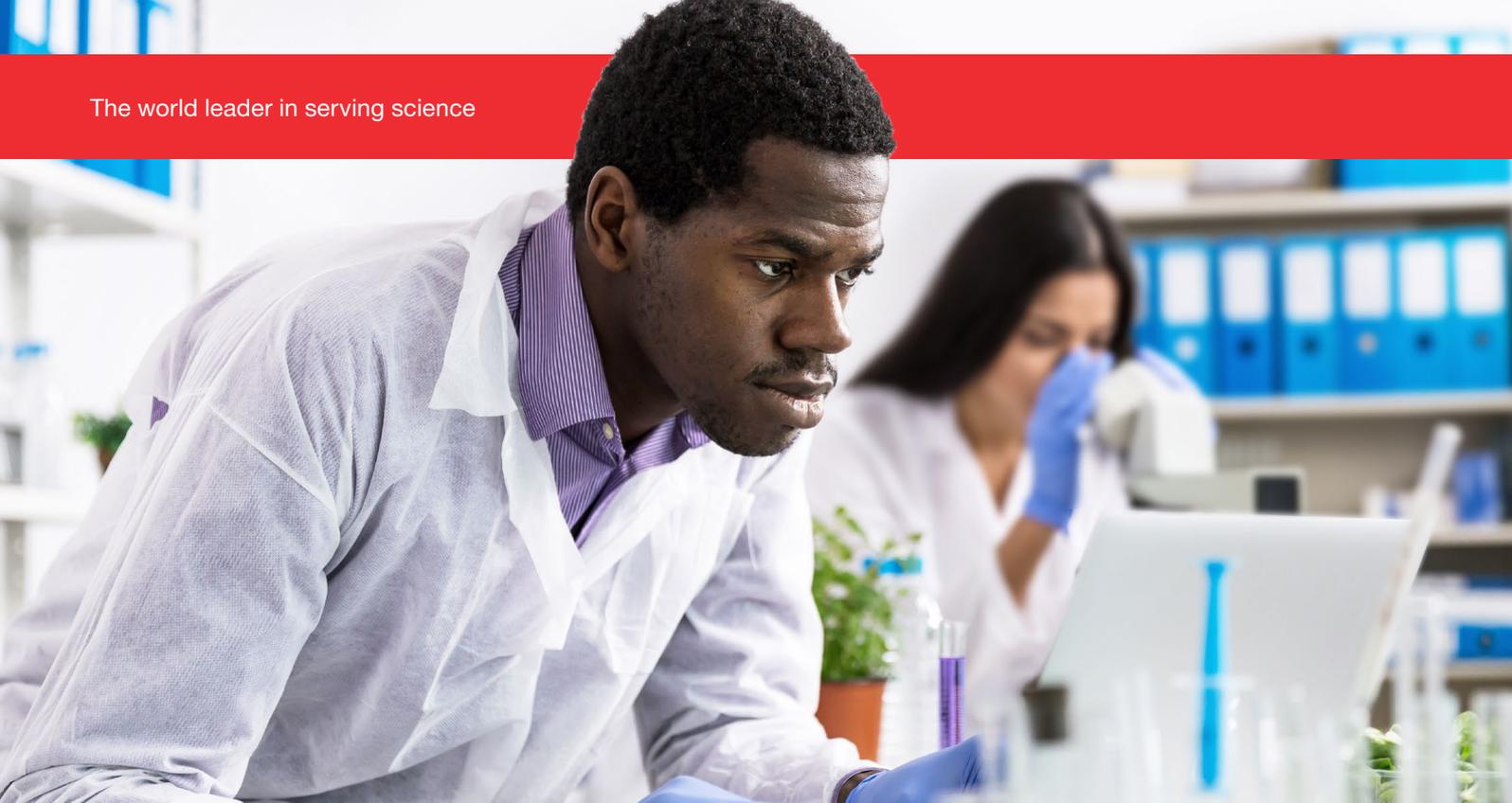
RNA-binding protein antibodies validated for RNA immunoprecipitation

50 μ m

invitrogen

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Thermo Fisher Scientific Antibody Scholarship Program

The Thermo Fisher Scientific Antibody Scholarship Program awards scholarship funds to deserving undergraduate and graduate students in a field of biological research. The goal of this program is to ensure that well-educated students with a passion for science and learning are prepared for future opportunities in scientific research, teaching, and technological development.

Congratulations to the 2019 Antibody Scholarship winners

Azadeh Hadadianpour—Vanderbilt University

Isha Puri—Harvard University

Colin J. Mann—University of California San Diego

Salil Uttarwar—Washington University in St. Louis

Stella Paffenholz—Gerstner Sloan Kettering
Graduate School of Biomedical Sciences

Student athlete*—California Institute of Technology

Get more information and see the profiles of the award winners
at thermofisher.com/antibodyscholarship

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S C I E N T I F I C

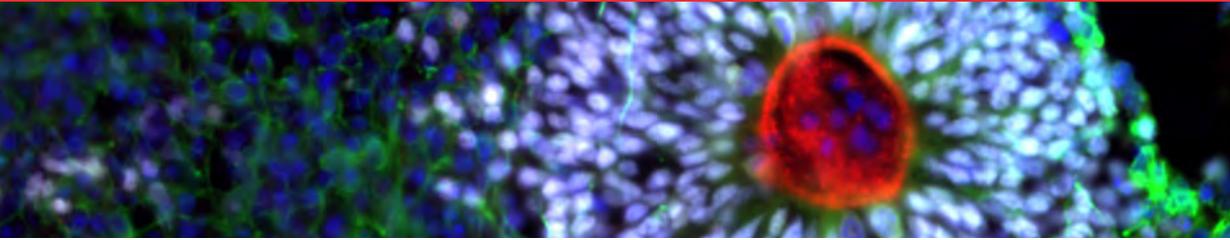
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Scholarship award eligibility criteria

No purchase necessary to apply for or receive a scholarship. The Program was open only to graduate and undergraduate students who (i) are attending an accredited US college or university in the Fall 2019 semester; (ii) are legal residents of one of the 50 US states or the District of Columbia; (iii) are at least 18 years of age as of August 2, 2019; and (iv) have a declared major of biology, chemistry, biochemistry, or a related life science field.

*Name withheld due to NCAA rules.

Health Care Professionals (term defined in the Official Rules) may not participate in this Program. US federal or state government employees may not participate in this Program. This Program is void where prohibited by law. A committee selected scholarship recipients whom the committee determined best embody one or more of Sponsor's values of Integrity, Intensity, Innovation, and Involvement, as demonstrated by the Application. All scholarship award payments will be made directly to the college or university at which the recipient is enrolled. The complete Official Rules are available at thermofisher.com/antibodyscholarship. In case of any inconsistency between these abbreviated rules and the complete Official Rules, the complete Official Rules will always prevail.



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Request the poster “5 steps to multiplexed fluorescent western blotting”

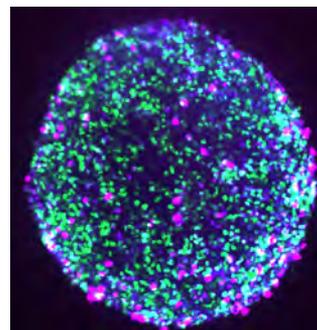
Now available upon request, the “5 steps to multiplexed fluorescent western blotting” poster features tips for optimizing each step of multiplexed western blotting to achieve publication-quality results. Each step in the workflow—from protein sample preparation, to electrophoresis, transfer, antibody incubation, and imaging—is covered on this 24 x 20 inch poster. You can find advice on antibody and fluorophore selection, as well as links for downloading helpful application articles. The reverse side offers troubleshooting tips for avoiding pitfalls that can produce less-than-perfect blots. Request this poster at thermofisher.com/5steps-multiplexwesterns.



Spheroid cell culture: Practical solutions for frequently asked questions

3D cell models—including spheroids, tumor spheroids or tumoroids, and organoids—have become important experimental tools for cancer biology and immunology, as well as for drug screening, discovery, and development. 3D models are relevant for many different application areas because their microenvironment can resemble the microanatomy of *in vivo* systems better than traditional 2D models. The formation and growth of these 3D cell models, however, can be more difficult because of the increased complexity that comes with spheroid culture.

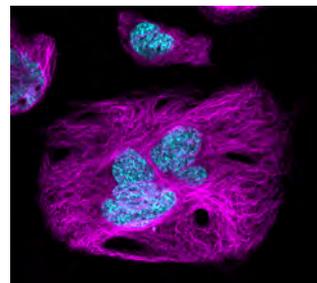
Spheroid size and uniformity across culture wells and plates and the expression of subcellular molecular markers are key concerns that can be addressed with the appropriate cultureware, media, fluorescent reagents and assays, and instrumentation. Thermo Fisher Scientific offers Invitrogen™, Gibco™, and Thermo Scientific™ products across the entire 3D workflow, in addition to resources, tip and tricks, and support for frequently asked questions. Find out more at thermofisher.com/5steps3d.



Watch the on-demand webinar “Best practices: 5 steps to live-cell imaging”

Fluorescence imaging of live cells is a powerful approach for the study of dynamic cellular processes and events. Recent advances in fluorescent dye development and imaging technology have led to the application of live-cell imaging in diverse fields, including developmental and stem cell biology, drug discovery, and environmental studies.

The webinar “Best practices: 5 steps to live-cell imaging” from Thermo Fisher Scientific summarizes what we have learned from over 40 years of cell imaging research and expertise. Designed for those new to live-cell imaging as well as experienced researchers, this webinar demonstrates how to obtain publication-quality live-cell images routinely and efficiently, while minimizing waste and avoiding costly mistakes. We also discuss long-proven tools and protocols for achieving the best possible results in your live-cell imaging workflow. Access this on-demand webinar at thermofisher.com/5steps-live.



Download this *Nature* booklet to learn more about antibody validation* and reproducibility

In collaboration with *Nature*, we have compiled a series of 3 articles that explore the antibody reproducibility crisis, antibody protocols and standards, and Thermo Fisher Scientific's specificity testing methodology, including data acquired using numerous antibodies against proteins in a variety of signaling pathways. Find out about the stringent measures Thermo Fisher Scientific uses to ensure that its antibodies are consistently of the highest quality—request to download this 8-page booklet at thermofisher.com/antibodybooklet.

*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.

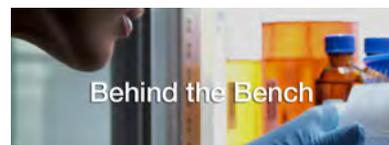


Behind the Bench blog: What's new with antibodies

We are continually updating our Invitrogen™ antibody portfolio with verified primary antibodies for use in flow cytometry, IF/ICC/IHC, western blotting, and ELISAs. See what's new in 2 blog posts:

- "Let's get 'specific' about the TNFR pathway!" focuses on the TNFR pathway and recommends CRISPR-validated Invitrogen antibodies for key proteins in this pathway
- "DIY neurons for antibody validation" describes our use of stem cells to generate several different types of neuron progenitor cells, which can then be employed for antibody validation*

Find these posts and more at thermofisher.com/blog/behindthebench. Or go directly to our antibody search tool at thermofisher.com/antibodies.



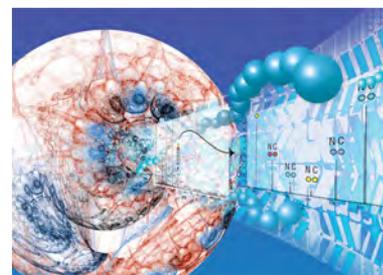
5th Annual Thermo Scientific Tandem Mass Tag (TMT) Research Awards: Winners announced!

Isobaric chemical tags are powerful tools that enable multiplexed identification and quantitation of thousands of proteins from different samples, using tandem mass spectrometry (MS/MS). Tandem mass tag (TMT) labeling reagents contain up to 11 different isobaric compounds for labeling protein samples in parallel and then combining them for analysis on high-resolution MS/MS platforms such as the Thermo Scientific™ Orbitrap Fusion™ Lumos™ series, Orbitrap Elite™ device, and Q Exactive™ series, each supported by Proteome Discoverer™ 2.2 Software.

We are pleased to announce this year's recipients of the Thermo Scientific™ TMT Research Awards, given annually to 3 researchers selected by a panel of judges from Thermo Fisher Scientific and Proteome Sciences in recognition of the innovation and potential impact of their use of TMT labeling reagents in conjunction with other Thermo Scientific™ MS reagents. These recipients were recognized at the 2019 American Society for Mass Spectrometry (ASMS) annual meeting:

- Gold Award—Dr. Amirata Saei Dibavar, Karolinska Institute, Sweden
- Silver Award—Dr. Anja Andrejeva, Cambridge Centre for Proteomics, University of Cambridge, UK
- Bronze Award—Dr. Paula Díez, Leiden University Medical Center, The Netherlands

Applications for the 6th Annual TMT Research Awards are available at thermofisher.com/tmtgrant.



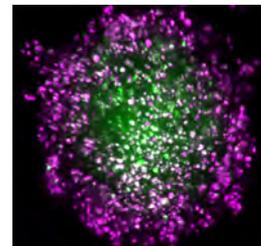
SYTOX Deep Red and SYTO Deep Red stains for nuclear labeling

The Invitrogen™ SYTOX™ Deep Red and SYTO™ Deep Red nucleic acid stains specifically label the cell nucleus with an intensely bright deep red fluorescence. Due to their long-wavelength emission, these dyes can be detected in the far-red fluorescence channel using microscopes, fluorimeters, microplate readers, and flow cytometers, and they can be multiplexed with blue, green, orange, red, and near-infrared fluorophores.

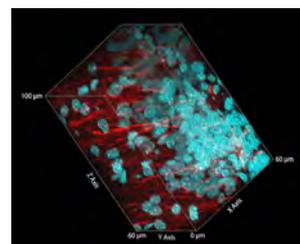
SYTOX Deep Red stain (Ex/Em = 660/682 nm) is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes but does not cross the membranes of live cells. Thus, SYTOX Deep Red stain is useful for staining the nuclei of fixed or dead cells, particularly in ICC, IHC, and IF experiments. SYTO Deep Red Nucleic Acid Stain (Ex/Em = 652/669 nm) is a cell-permeant dye that can cross cell membranes and stain the nuclei of live, dead, and fixed cells.

After a brief incubation with cells, these dyes exhibit bright fluorescence with excellent photostability, making them ideal for quantitative, single-step nuclear labeling. Both dyes exhibit increased fluorescence in response to increasing concentrations of dsDNA, and only minimal signal in the presence of RNA or ssDNA. To learn more about these and other nuclear stains, visit thermofisher.com/image-the-nucleus.

| Product | Quantity | Cat. No. |
|--|--------------------|------------------|
| SYTO™ Deep Red Nucleic Acid Stain, for live cells | 1 vial 5 vials | S34900 S34901 |
| SYTOX™ Deep Red Nucleic Acid Stain, for fixed/dead cells | 50 µL 5 x 50 µL | S11380 S11381 |



Spheroid labeling. Niclosamide-treated spheroids were stained with Invitrogen™ SYTO™ Deep Red Nucleic Acid Stain (Cat. No. S34900) and Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent (Cat. No. C10723).



Fixed-cell labeling. A 100 µm FFPE rat brain section was immunostained for tubulin (red) and counterstained with Invitrogen™ SYTOX™ Deep Red Nucleic Acid Stain (cyan, Cat. No. S11380).

Image-iT plant tissue clearing and mounting reagents

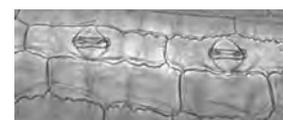
Plant cells and tissues have structures with different refractive indexes, causing light scattering and spherical aberrations that can result in opaqueness during imaging. Thermo Fisher Scientific is pleased to offer the Invitrogen™ Image-iT™ Plant Tissue Clearing Reagent and Image-iT™ Hard-Set Mountant (based on Visikol™ for Plant Biology™ and Visikol™ Mount™ reagents), which are compatible with both fluorescence-based and colorimetric applications.

Image-iT Plant Tissue Clearing Reagent, a soft-set mountant with a high refractive index of 1.45, renders plant tissue (e.g., whole leaf, root, shoot, etc.) optically clear for imaging. Image-iT Plant Tissue Hard-Set Mountant can then be used to mount and preserve the optically cleared tissue for imaging, storage, and archiving. Furthermore, Image-iT Plant Tissue Clearing Reagent is an effective replacement for chloral hydrate, traditionally used to clear plant tissues for microscopy.

| Product | Quantity | No. of slides | Refractive index | Cat. No. |
|--|----------|---------------|----------------------|----------|
| Image-iT™ Plant Tissue Clearing Reagent | 5 mL | 25 | 1.45 | V11329 |
| | 30 mL | 150 | | V11328 |
| | 100 mL | 500 | | V11327 |
| Image-iT™ Plant Tissue Hard-Set Mountant | 15 mL | 75 | 1.415 (after curing) | V11332 |
| | 30 mL | 150 | | V11331 |
| | 100 mL | 500 | | V11330 |



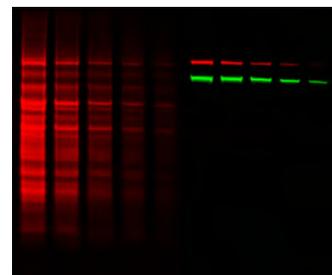
Fluorescence imaging. *Zea mays* L. leaf cells were fixed with Invitrogen™ Image-iT™ Fixative Solution (Cat. No. R37814), stained with Invitrogen™ SYTOX™ Deep Red Stain (Cat. No. S11380), incubated with 70% ethanol, optically cleared with Invitrogen™ Image-iT™ Plant Tissue Clearing Reagent (Cat. No. V11328) for 30 min, then mounted using Invitrogen™ Image-iT™ Plant Tissue Hard-Set Mountant (Cat. No. V11331).



Colorimetric imaging. The morphology of *Zea mays* L. leaf epidermis was observed after clearing tissue and mounting with Invitrogen™ Image-iT™ Plant Tissue Clearing Reagent (Cat. No. V11328) and Hard-Set Mountant (Cat. No. V11331). Epidermis cells and guard cells are easily identified.

No-Stain Protein Labeling Reagent for accurate and reliable total-protein normalization on western blots

When performing quantitative western blotting, researchers typically use housekeeping proteins to normalize their data; however, their expression can vary with experimental conditions, and they often produce oversaturated western blot signals. The Invitrogen™ No-Stain™ Protein Labeling Reagent offers a convenient, more accurate alternative for normalization. The No-Stain reagent detects all proteins and is applied to the membrane after transfer, allowing your choice of protein gel. It forms covalent bonds with proteins on membranes (or in gels you don't plan to transfer) within 10 minutes, does not require destaining, and can instantly be visualized using imagers equipped with a UV light source, 488 nm laser, or green LED such as the Invitrogen™ iBright™ FL1500 Imaging System. The No-Stain reagent has a broad linear detection range (1–80 µg protein/lane, and down to 20 ng protein/band) and is compatible with chemiluminescent and fluorescent immunodetection. Request a sample at thermofisher.com/no-stain.



Fluorescence western blot imaging. Visualization of total protein (left) using the Invitrogen™ No-Stain™ Protein Labeling Reagent (Cat. No. A44717) and specific proteins (right) using fluorescent antibodies.

| Product | Quantity | Cat. No. |
|------------------------------------|--------------|----------|
| No-Stain™ Protein Labeling Reagent | 10 reactions | A44717 |
| | 40 reactions | A44449 |

Introducing the iBright 1500 Series Imaging Systems

The Invitrogen™ iBright™ CL1500 and iBright™ FL1500 Imaging Systems are the latest innovation in western blot imaging solutions for life science researchers. Both iBright 1500 systems can be used to image chemiluminescent and colorimetric western blots, fluorescent nucleic acid gels, fluorescent and colorimetric protein gels, and more; the iBright FL1500 system extends this functionality with added fluorescent western blot imaging support. Each model utilizes a powerful high-resolution 9.1-megapixel camera that is capable of detecting subtle differences in samples, as well as automatic exposure, zoom, focus, and sample rotation to speed up the image capture process. For more information, visit thermofisher.com/ibright.



iBright FL1500 Imaging System.

| Product | Quantity | Cat. No. |
|--------------------------------|----------|----------|
| iBright™ FL1500 Imaging System | 1 system | A44241 |
| iBright™ CL1500 Imaging System | 1 system | A44240 |

Pro-Detect Rapid Assay Kits for rapid determination of recombinant protein expression

Each step of the protein expression and purification workflow can be time-consuming, taking days to weeks to complete. Moreover, traditional methods used to confirm protein expression require significant hands-on time. Thermo Scientific™ Pro-Detect™ Rapid Assay Kits enable quick and efficient detection of tagged recombinant proteins without the need to perform complex assays such as ELISA or western blotting. Pro-Detect Rapid Assay Kits are single-use, dipstick-style lateral flow strips that provide qualitative confirmation of expressed fusion-tag proteins in 10–15 minutes. These rapid test kits can detect a specific protein tag of interest—e.g., His, GST, Myc, and Fc—directly from cell culture media, cell lysates, or purified protein solutions, and display the results visually without the need for specialized equipment. Learn more at thermofisher.com/pro-detect.

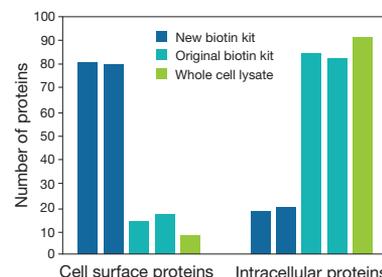


Pro-Detect Rapid Assay Kit.

Improved method for cell-surface protein biotinylation and isolation

Cell-surface proteins represent a key subset of cellular proteins and play major roles in signal transduction, cell adhesion, and ion transport. These proteins are challenging to efficiently extract and isolate due to their plasma membrane-spanning domains. Often the harsh detergents and conditions necessary for efficient extraction result in denaturation and contamination of plasma membrane proteins after isolation.

The new, improved Thermo Scientific™ Pierce™ Cell-Surface Protein Biotinylation and Isolation Kit utilizes membrane-impermeant, amine-reactive sulfo-NHS-SS-biotin to efficiently label cell-surface proteins that contain accessible lysines, enabling the selective biotinylation, solubilization, and enrichment of plasma membrane proteins. This new kit outperforms the now-discontinued Thermo Scientific™ Pierce™ Cell-Surface Protein Isolation Kit (see figure for comparison). Learn more about protein purification and isolation at thermofisher.com/membraneprotein.

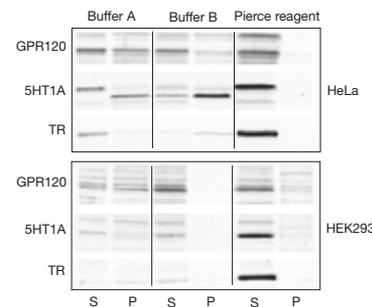


Classification of the top 100 most abundant proteins, as identified by mass spectrometry. When compared with whole cell lysate or the discontinued Thermo Scientific™ Pierce™ Cell-Surface Protein Isolation Kit, the new Thermo Scientific™ Pierce™ Cell-Surface Protein Biotinylation and Isolation Kit (Cat. No. A44390) improves the enrichment of cell-surface proteins while reducing background.

| Product | Quantity | Cat. No. |
|--|-----------|----------|
| Pierce™ Cell-Surface Protein Biotinylation and Isolation Kit | 8 samples | A44390 |

Specialized reagent for GPCR extraction and stabilization

G protein-coupled receptors (GPCRs), which are members of a large family of transmembrane proteins, play a key role in transmitting signals across the cell membrane and are potential biomarkers and therapeutic targets for cancers and immune system disorders. One major challenge in GPCR research is the efficient extraction of the receptor from the cell membrane without compromising the structural and functional integrity of the protein. The Thermo Scientific™ Pierce™ GPCR Extraction and Stabilization Reagent is the first and only commercially available reagent designed specifically for GPCRs found in mammalian cells or tissue. The resulting whole cell lysate contains solubilized and stabilized receptors. Learn more about protein purification and isolation at thermofisher.com/membraneprotein.



GPCR extraction. Gel electrophoresis analysis shows that, compared with other reagents for preparing whole lysates, the Thermo Scientific™ Pierce™ GPCR Extraction and Stabilization Reagent (Cat. No. A43436) produces more consistent banding. S = solubilized fraction; P = insoluble pellet.

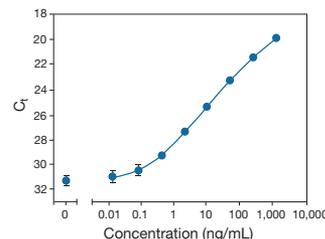
| Product | Quantity | Cat. No. |
|---|----------|----------|
| Pierce™ GPCR Extraction and Stabilization Reagent | 100 mL | A43436 |

New ProQuantum immunoassay targets

Invitrogen™ ProQuantum™ immunoassays are a platform innovation that provides an affordable, no-wash, high-performance assay using a qPCR instrument. ProQuantum assays provide:

- High sensitivity and broad dynamic range
- Low sample consumption—only 2–5 μL of sample needed to obtain results in triplicate
- A fast, easy workflow—2 hours from sample to answer, with no wash steps
- Intuitive software for comprehensive data analysis and statistical groupwise comparison

See available targets (including new adiponectin, IgE, IgG4, IgM, and IL-17A), or learn more about Custom Assay Development services at thermofisher.com/proquantum.



Standard curve for the ProQuantum Human IgG4 Immunoassay Kit. Assay range is 0.08–1,250 ng/mL, with a limit of detection (LOD) of <0.05 ng/mL.

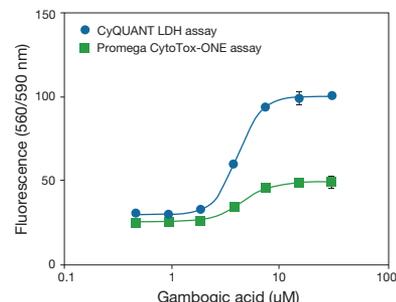
CyQUANT LDH Cytotoxicity Assay: Now a fluorescence-based assay

The new Invitrogen™ CyQUANT™ LDH Cytotoxicity Assay is a fluorescence-based microplate assay that provides a simple and reliable method for determining cytotoxicity. Lactate dehydrogenase (LDH) is a cytosolic enzyme present in many different cell types that is released into the culture medium upon damage to the plasma membrane. The CyQUANT LDH Cytotoxicity Assay provides the reagents to accurately and quantitatively measure this extracellular LDH using a sample of the culture medium; no cells are consumed in this assay. LDH activity is measured using a coupled reaction that ultimately reduces resazurin to resorufin, and this kit utilizes our recently developed, highly purified resazurin that provides increased assay sensitivity and lower background signals. This new fluorescence-based CyQUANT LDH assay is:

- Convenient—add-mix-read assay format for adherent and suspension cells and 3D cell models
- Accurate—quantitative, reproducible measurement of LDH release
- Flexible—ideal for high-throughput screening and studies of cytotoxicity over time
- Robust—formulated with highly purified resazurin, which provides a large assay dynamic range

Learn more at thermofisher.com/cyquant-ldh.

| Product | Quantity | Cat. No. |
|---|--------------|----------|
| CyQUANT™ LDH Cytotoxicity Assay, fluorescence | 200 assays | C20302 |
| | 1,000 assays | C20303 |



The CyQUANT LDH Cytotoxicity Assay, fluorescence, provides a large dynamic range. A549 cells exposed to gambogic acid were assayed using the Invitrogen™ CyQUANT™ LDH Cytotoxicity Assay, fluorescence (Cat. No. C20302) or the Homogeneous Membrane Integrity Assay (Promega), according to manufacturers' protocols. Both assays generated overlapping IC₅₀ values, but the fluorescent signal was approximately 2-fold greater for the CyQUANT LDH assay.

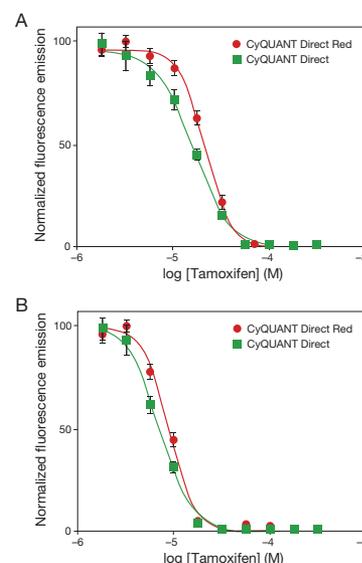
CyQUANT Direct Red Cell Proliferation Assay: An add-and-read assay, now in red

The Invitrogen™ CyQUANT™ Direct Red Cell Proliferation Assay is a simple fluorescence-based microplate assay that can be used to assess cell viability and growth or compound toxicity. The assay consists of a cell-permeant DNA-binding dye that fluoresces red when bound to DNA (in all cells), and a cell-impermeant fluorescence background suppressor that only enters cells with compromised membranes. The original CyQUANT Direct assay detects changes in proliferation by emitting green fluorescence, which prevents this assay from being multiplexed with GFP-expressing cells or those labeled with green-fluorescent reagents. In contrast, the CyQUANT Direct Red assay emits a red-fluorescent signal that can be easily multiplexed with spectrally distinct probes. Designed for use with microplate readers, the CyQuant Direct Red assay is:

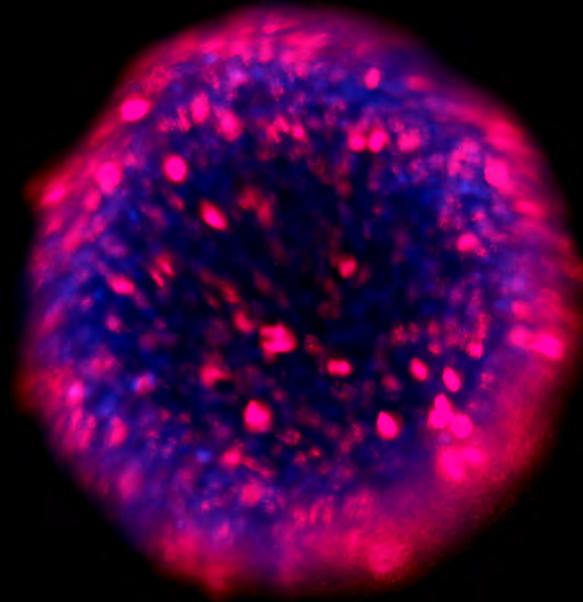
- Convenient—no washes, cell lysis, or temperature equilibration; simply add and read
- Accurate—measurements are based on amount of cellular DNA, which is highly regulated and independent of the metabolic state of the cells
- Robust—optimized reagents result in a highly sensitive assay with a large dynamic range
- Stable—red-fluorescent signal is stable for 7 hours, ideal for large screening assays

Learn more at thermofisher.com/cyquant-direct.

| Product | Quantity | Cat. No. |
|--|-----------------|----------|
| CyQUANT™ Direct Red Cell Proliferation Assay | 10 microplates | C35013 |
| | 100 microplates | C35014 |
| CyQUANT™ Direct Cell Proliferation Assay | 10 microplates | C35011 |
| | 100 microplates | C35012 |



CyQUANT Direct cell proliferation assays produce very similar screening results. (A) A549 and (B) HCASMC cells were assayed using the Invitrogen™ CyQUANT™ Direct Red Cell Proliferation Assay (Cat. No. C35013) or CyQUANT™ Direct Cell Proliferation Assay (Cat. No. C35011), and the resulting IC₅₀ values were very similar.



Elucidate the underlying mechanisms of Parkinson's disease and other neurological disorders

Fixed and live cell research tools for neuronal protein and structural analyses.

Parkinson's disease (PD) is a debilitating, chronic, and progressive neurological disease that affects around 10 million people worldwide. PD is accompanied by loss of motor function and, without a curative treatment, care remains palliative. Early diagnosis of PD and similar neurodegenerative diseases is difficult because of their common symptoms and pathophysiology and the lack of understanding of their distinguishing molecular characteristics. Thermo Fisher Scientific is committed to developing research tools for neuroscientists investigating the molecular mechanisms of PD and other neurodegenerative diseases using *in vitro*, *ex vivo*, and *in vivo* experimental systems.

Figure 1 (above). Tubulin Tracker Deep Red as a marker of neurosphere maturation. Neural stem cell (NSC)-derived neurospheres were cultured using Gibco™ Neurobasal™ Plus Medium (Cat. No. A3582901) with Gibco™ CultureOne™ Supplement (Cat. No. A3320201) in Thermo Scientific™ Nunc™ Sphera™ 96 U-Well Microplates. The neurospheres were then stained with Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605) and Invitrogen™ Tubulin Tracker™ Deep Red (Cat. No. T34077) to assess neurosphere maturation. Image was generated using an Invitrogen™ EVOS™ FL Auto 2 Imaging System with a 20x objective and an Invitrogen™ EVOS™ Cy®5 Light Cube.

In general the etiology of PD and many other neurodegenerative disorders, including Alzheimer's disease (AD), is poorly understood. The loss of motor function associated with PD has led researchers to investigate the degeneration of dopaminergic neurons in the substantia nigra [1,2]. Key molecular mechanisms implicated in this neurodegeneration can be broadly classified into categories that include mitochondrial dysfunction, genetic mutations, and defects in protein clearance pathways that lead to protein aggregation, formation of neuronal inclusion bodies, and ultimately neuronal deterioration [2-5].

Historically, diagnosis has relied upon the identification of protein aggregates (e.g., Lewy bodies, in the case of PD) that occur in the later stages of the disease. Neuronal inclusion body formation, defined by the presence of these insoluble protein aggregates, is responsible for the breakdown of neuronal microtubules, a causative event of neuronal diseases [1-5]. Two proteins commonly identified in neurodegenerative diseases—also referred to as proteinopathies due to protein misfolding—are tau and synuclein [5]. The proteinopathy of PD is primarily attributed to alpha-synuclein, the main structural component of Lewy bodies in nerve cells, which are a defining characteristic of not only PD but also Lewy body dementia (LBD) and other disorders. Although tau aggregation and the formation of amyloid plaques have been more extensively described in AD, tau has also been linked with alpha-synuclein as an interdependent partner in the progression of PD [2-6].

Although the protein-protein interactions that influence formation of inclusion bodies and plaques have been studied at the molecular level, the underlying causes of the degenerative pathophysiology are still unknown. Obviously, there is a need for more appropriate

models and reagents to elucidate the principal mechanisms and indicators for the onset and progression of PD. In the first half of this article, we give an overview of some key proteins involved in PD and other neurodegenerative diseases, and the Invitrogen™ primary antibodies available →

Table 1. Recommended antibodies for detecting heat shock proteins (HSPs), from Thermo Fisher Scientific.

| Target protein | Cat. No. |
|--------------------------------------|------------------|
| HSP40 (polyclonal) | PA5-17382 |
| HSP60 (clone 4B9/89 or clone 2E1/53) | MA3-012, MA3-013 |
| HSP60 (polyclonal) | PA5-34760 |
| HSP70 (clone 4G4) | MA3-009 |
| HSP70 (clone 2A4) | MA3-008 |
| HSP90 alpha (clone 3B6) | MA3-010 |
| HSP90 beta (clone H9010) | 37-9400 |
| Phospho-HSP27 (Ser15, polyclonal) | PA1-018 |
| Phospho-HSP27 (Ser85, polyclonal) | PA1-005 |

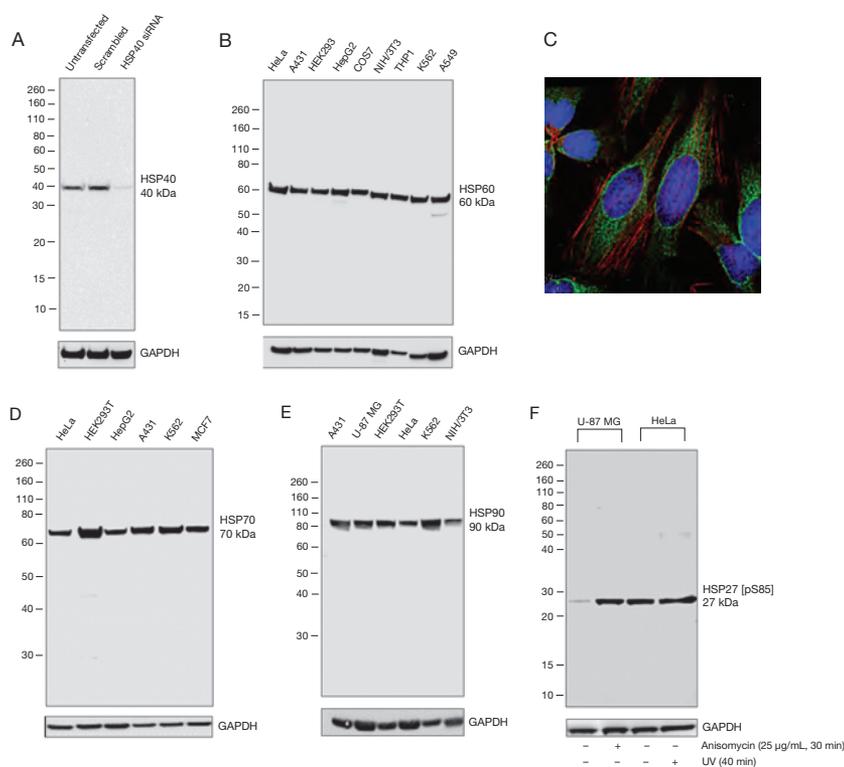


Figure 2. Assessment of heat shock protein (HSP) antibody specificity. (A) Western blot analysis of HSP40 antibody (Cat. No. PA5-17382) using RNAi. (B) Western blot analysis across multiple cell lines and (C) immunofluorescence analysis in HeLa cells using HSP60 antibody (clone 2E1/53, Cat. No. MA3-013). For the immunofluorescence analysis, HeLa cells were labeled with HSP60 antibody (green) and counterstained with rhodamine phalloidin (red, Cat. No. R415) and DAPI (blue, Cat. No. D1306). (D) Western blot analysis of HSP70 (clone 2A4, Cat. No. MA3-008) and (E) HSP90 (clone 3B6, Cat. No. MA3-010) antibodies across multiple cell lines. (F) Western blot analysis of phospho-HSP27 (Ser85) antibody (Cat. No. PA1-005) in cells subjected to anisomycin or UV, both known to activate HSPs. For the western blot analyses, primary antibodies were probed with Invitrogen™ Goat Anti-Rabbit IgG or Goat Anti-Mouse IgG Superclonal™ Recombinant Secondary Antibody, HRP (Cat. No. A27036 or Cat. No. A28177), detected using the Thermo Scientific™ Pierce™ ECL Western Blotting Substrate (Cat. No. 32106), and imaged using the Invitrogen™ iBright™ FL1000 Imaging System.

to study these targets (Figures 2–5, Tables 1–3). In the second half, we highlight recently developed fluorescent tubulin-selective probes that can be used in live neurons to further reveal the relationships between neuronal protein aggregation and microtubules, as well as the possible roles of this association in neuronal disease.

Antibody detection of molecular chaperones in PD

Several proteins have emerged as key players in signaling pathways associated with various stages of PD, including early onset, and late-stage progression. Cellular defense mechanisms inherent to neurons play a critical role in preventing protein aggregation and maintaining cellular homeostasis. Molecular chaperones, also known as heat shock proteins (HSPs), have gained attention in PD research for their protective nature against misfolded proteins, preventing their aggregation.

HSPs are constitutively expressed or inducibly regulated in a number of different cell types, and disruption in their function can quickly lead to the accumulation of misfolded proteins and eventually cell death. These proteins, therefore, are being investigated as a means both to understand PD disease biology and to study their potential role in disease treatment. The functions of several HSPs (including HSP27, HSP40, HSP60, HSP70, and HSP90) that are localized to axons or synapses and associated with alpha-synuclein are found to be compromised

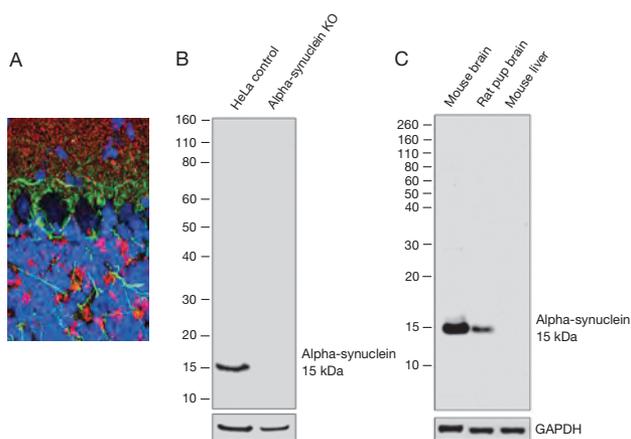


Figure 3. Assessment of alpha-synuclein antibody specificity. (A) Immunofluorescence analysis in mouse brain tissue using alpha-synuclein polyclonal antibody (red, Cat. No. PA5-17239) and a neurofilament-L monoclonal antibody (green); cells were counterstained with DAPI (blue). Western blot analysis of alpha-synuclein antibody using (B) CRISPR knockout (KO) of alpha-synuclein in HeLa cells and (C) relative expression of alpha-synuclein in brain and liver. Western blot detection was performed as described in Figure 2.

in PD [7-9]. Figure 2 shows the validation* data for representative Invitrogen™ HSP antibodies, and Table 1 provides a list of antibodies against several members of the HSP family.

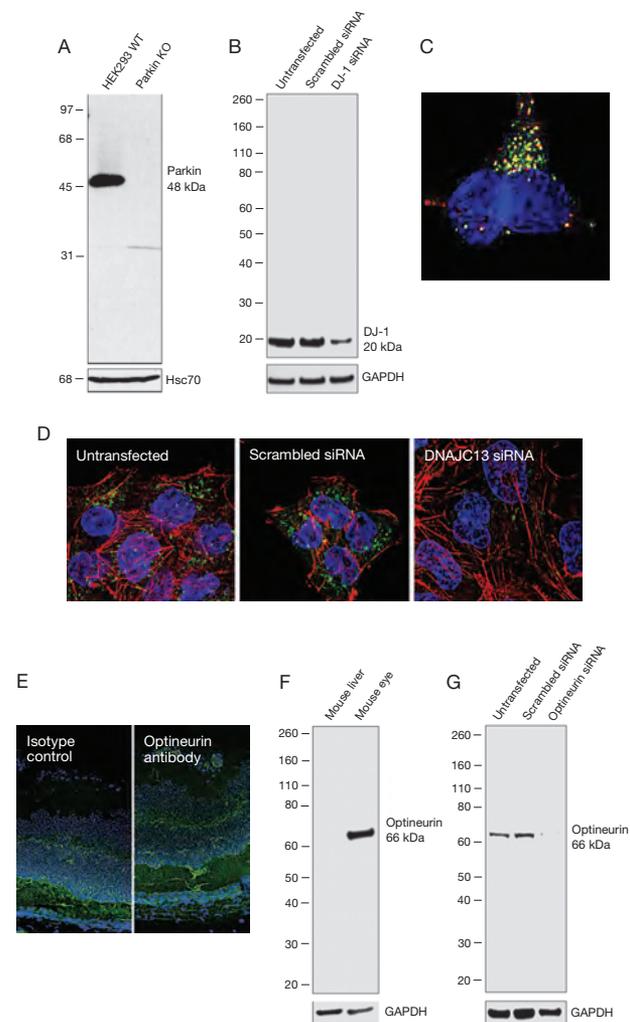


Figure 4. Assessment of antibody specificity. Western blot analysis of (A) parkin (clone 21H24L9, Cat. No. 702785) and (B) DJ-1 (Cat. No. PA5-13404) antibodies to show specificity using CRISPR knockout (KO) and RNAi, respectively. (C) Immunofluorescence analysis in HepG2 cells labeled with LIMP2 antibody (green; clone 22H6L14, Cat. No. 703037) and Invitrogen™ LysoTracker™ Red DND-99 (red, Cat. No. L7528); cells were counterstained with DAPI (blue). (D) Immunofluorescence analysis of DNAJC13 antibody (green; clone 11H13L5, Cat. No. 702773) in A431 cells using RNAi; cells were counterstained with rhodamine phalloidin (red) and DAPI (blue). (E) Immunofluorescence analysis of optineurin in mouse retina using an isotype control (green) or optineurin recombinant polyclonal antibody (green, Cat. No. 711879); cells were counterstained with DAPI (blue). Western blot analysis of the optineurin antibody in (F) liver and eye lysates and (G) optineurin siRNA-silenced NIH/3T3 cells. Western blot detection was performed as described in Figure 2.

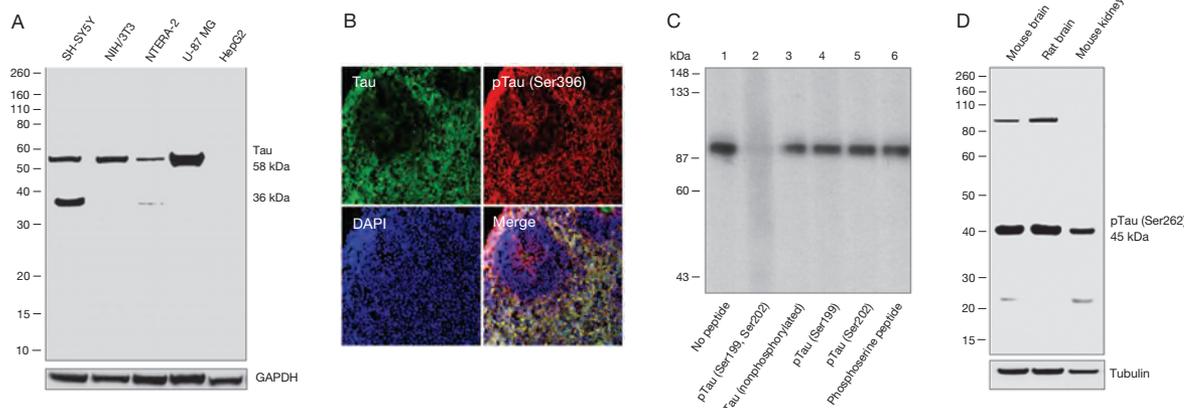


Figure 5. Assessment of tau antibody specificity. (A) Western blot analysis of tau antibody (clone TAU-5, Cat. No. MA5-12808) across multiple cell lines. (B) Immunofluorescence analysis in human iPSC-derived forebrain organoids at day 40 using tau antibody (green; clone TAU-5, Cat. No. AHB0042) and phospho-tau (Ser396) (red, Cat. No. 44-752G), and counterstained with DAPI (blue); data used with permission from Zhexing Wen, Emory University. (C) Western blot analysis of the specificity of phospho-tau (Ser199, Ser202) antibody (Cat. No. 44-768G) binding in the presence of the specific phosphopeptide target (lane 2), the corresponding nonphosphorylated peptide (lane 3), and other phosphoserine-containing peptides (lanes 4–6); signal loss was only seen with the specific peptide. (D) Western blot analysis of phospho-tau (Ser262) antibody (Cat. No. 44-750G) in mouse brain, rat brain, and mouse kidney lysates. For the western blot analyses, primary antibodies were probed with Invitrogen™ Goat Anti-Rabbit IgG or Goat Anti-Mouse IgG Secondary Antibody, HRP (Cat. No. G21234 or Cat. No. 62-6520), detected using the Thermo Scientific™ Pierce™ ECL Western Blotting Substrate (Cat. No. 32106), and imaged using the Invitrogen™ iBright™ FL1000 Imaging System.

Antibody detection of aggregation markers in PD

The accumulation of alpha-synuclein, one of the main structural components of Lewy bodies, is a hallmark of the latter stages of PD [10]. Although its specific role in the neuronal degeneration process is not fully understood, studies have suggested that it is involved in aggregation of misfolded protein that eventually converges upon dopaminergic neurons in the form of Lewy bodies [6, 10, 11]. Figure 3 demonstrates validation data for the Invitrogen™ alpha-synuclein antibody, which was tested using different parameters to ensure its specificity.

A growing number of genes associated with PD are related to mitochondrial function and oxidative stress, emphasizing the role of mitochondria in neuronal degeneration and disease progression [12]. Similarly, impairment of lysosome-mediated protein degradation via autophagy or endocytosis directly impacts the ability of neurons to clear alpha-synuclein aggregates in Lewy bodies [13, 14].

Table 2. Recommended antibodies for Parkinson's disease research, from Thermo Fisher Scientific.

| Target protein | Protein function | Cat. No. |
|--------------------|--|-----------|
| Ataxin3 | Protein clearance | 711823 |
| DENND5A | Vesicle-mediated transport and RAB-activating guanine nucleotide exchange factor (GEF) | 702789 |
| DJ-1 (Park7) | Molecular chaperone | PA5-13404 |
| DNAJC13 (RME-8) | Membrane trafficking | 702773 |
| DNML1 | Clathrin-mediated endocytosis | 702782 |
| LIMP2 | Lysosomal membrane protein | 702770 |
| Mitofusin-2 (MFN2) | Mitochondrial maintenance | 711803 |
| Optineurin | Membrane trafficking | 711879 |
| Alpha-synuclein | Regulation of neurotransmitter release | PA5-17239 |
| TMEM230 | Synaptic vesicle recycling | 702792 |
| VAMP1 | Vesicular transport | 702787 |

Table 3. Recommended tau antibodies, from Thermo Fisher Scientific.

| Target protein | Cat. No. |
|--|--|
| Tau (clone HT7) | MN1000, MN1000B (biotin conjugate) |
| Tau (clone TAU-5) | AHB0042, MA5-12808, MA5-12805 (biotin conjugate) |
| Tau (clone T46) | 13-6400 |
| Phospho-tau (Ser199, Ser202) | 44-768G |
| Phospho-tau (Ser202, Thr205) (clone AT8) | MN1020, MN1020B (biotin conjugate) |
| Phospho-tau (Thr231) (clone AT180) | MN1040 |
| Phospho-tau (Thr212, Ser214) (clone AT100) | MN1060 |

Several proteins involved in normal mitochondrial or lysosomal functioning, such as parkin, LIMP2, and PINK1 [15], are found to be mutated in PD. As a result, affected cells are subjected to increased oxidative stress and eventually cell death. Additionally, mutations in proteins →

such as DNAJC13 (required for receptor-mediated endocytosis) and optineurin (important for maintenance of the Golgi complex and membrane trafficking) have been identified as contributing to PD pathology [16,17]. In Figure 4, we showcase representative validation data for antibodies against several of these targets that can be used across applications, including immunofluorescence and western blotting. Table 2 provides a list of antibodies against a variety of important PD targets.

The link between alpha-synuclein and tau in PD

Tau, a member of the microtubule-associated protein family (MAPT), is associated with microtubule stabilization, membrane binding, and axonal transport [17-20]. Under normal physiological conditions, tau is soluble and unfolded; however, under pathological conditions in PD or AD, misfolding of tau leads to insoluble neurofibrillary tangles (NFTs)—a pathological effect called a tauopathy [4-6,21]. In such pathology, hyperphosphorylation of tau causes protein aggregation and accumulation, which in turn damages neurons and disrupts axonal transport [17,18,21]. Although alpha-synuclein dysregulation is a possible cause of PD, it has recently been reported that both alpha-synuclein and tau are present in the same Lewy body aggregate in many postmortem brains with PD and dementia [4-6]. Consequently, clinicians and researchers alike are focusing on the link between tau and alpha-synuclein in PD at the molecular level [4-6,10]. Invitrogen primary antibodies against total tau as well as several important phosphorylated

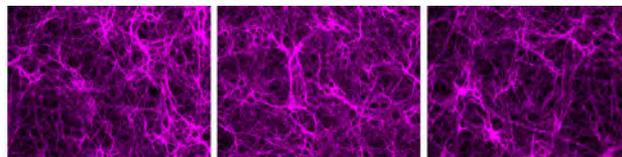


Figure 6. Live rat cortical neurons labeled with Tubulin Tracker Deep Red. Gibco™ Primary Rat Cortex Neurons (Cat. No. A1084001) were prepared and grown using the Gibco™ B-27™ Plus Neuronal Culture System (Cat. No. A3653401) and Gibco™ GlutaMAX™ Supplement (Cat. No. 35050061) for 14 days. Cells were then stained with Invitrogen™ Tubulin Tracker™ Deep Red (Ex/Em = 652/669 nm, Cat. No. T34077) and imaged in Invitrogen™ Live Cell Imaging Solution (Cat. No. A14291DJ) on an Invitrogen™ EVOS™ FL Auto 2 Imaging System with a 20x objective and an Invitrogen™ EVOS™ Cy®5 Light Cube.

forms have been published and highly cited in publications. Figure 5 shows representative validation data for some of these antibodies, and Table 3 provides a list of antibodies against these important PD targets.

Relationship between tau and the microtubule cytoskeleton

Recently, tau aggregation and spreading into naive neurons has been shown to occur through tunneling nanotubes (TNTs) [8,18,20,21]. There are different types of TNTs that are thought to provide cell-to-cell communication, and one type of TNT is composed of actin and microtubules [22]. Microtubules are highly dynamic polymers of tubulin that are important for neuronal outgrowth, morphology, and migration,

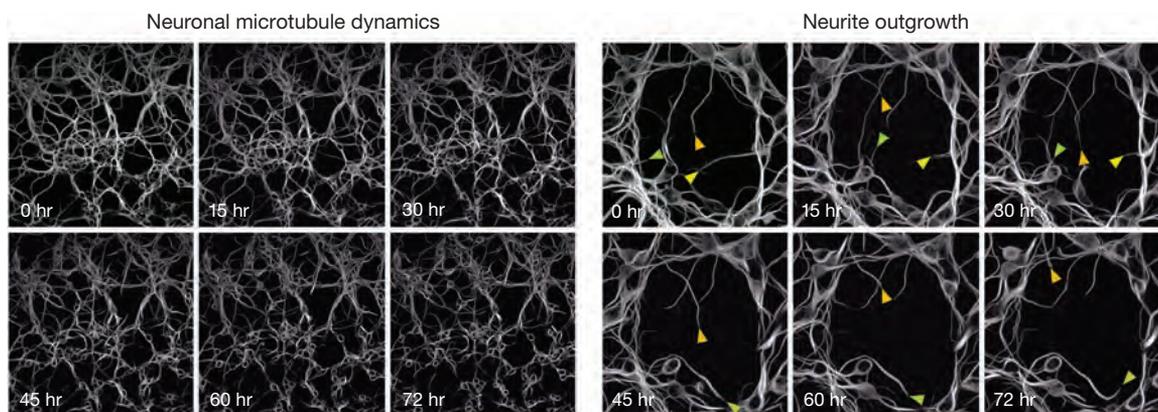


Figure 7. Tubulin Tracker Deep Red for extended time-lapse imaging in primary neurons. Gibco™ Primary Mouse Hippocampal Neurons (Cat. No. A15587) were cultured for 14 days *in vitro* using the Gibco™ B-27™ Plus Neuronal Culture System (Cat. No. A3653401) and Gibco™ GlutaMAX™ Supplement (Cat. No. 35050061) and then imaged across 72 hr at 5 min intervals. Left panel shows no changes in neuronal dynamic assembly, even after 72 hr of staining in Invitrogen™ Tubulin Tracker™ Deep Red (Cat. No. T34077). Right panel shows a 4-fold higher magnification of the neurons to monitor potential changes in neuronal outgrowth with dye treatment; arrows denote outgrowth activity, with each color tracking an individual neuron over time. Tubulin Tracker Deep Red was added to the culture at the beginning of the experiment; no wash step was required throughout the study. The labeled neurons were imaged in a live-cell chamber on a confocal laser-scanning microscope.

and there is growing evidence that their dysfunction is related to PD [21,23,24]. The relationships between alpha-synuclein and tau, as well as between tau and the microtubule cytoskeleton, present an opportunity to better characterize the mechanisms involved in protein aggregate formation in PD.

Tracking the microtubule cytoskeleton in live neurons

To visualize dynamic microtubules in live neuronal cultures, we offer the tubulin-selective fluorescent probes Invitrogen™ Tubulin Tracker™ Deep Red and Tubulin Tracker™ Green. These are cell-permeant fluorescent dyes that bind to polymerized tubulin within the microtubule. The Tubulin Tracker Green reagent (Ex/Em = 494/522 nm) has been used for the study of microtubule dynamics in Parkinson's disease-related research [18]. Neuronal staining using the recently introduced Tubulin Tracker Deep Red reagent (Ex/Em = 652/669 nm) is shown in Figures 6–8.

Time-lapse imaging in neurons

To test and demonstrate the low cytotoxicity and phototoxicity of Tubulin Tracker Deep Red, mouse embryonic hippocampal neurons were labeled with the tubulin-selective fluorescent reagent and microtubule formation in neuronal cultures was monitored over the course of 72 hours (at 5-minute intervals) using extended time-lapse imaging (Figure 7). Normal levels of dynamic assembly, outgrowth, and retraction of neurites were observed throughout the entire time course, with no detachment from the growth substrate or indication of other cytotoxic phenotypes, even under aggressive imaging conditions. Given the relative sensitivity of primary neuronal cultures, these data clearly demonstrate

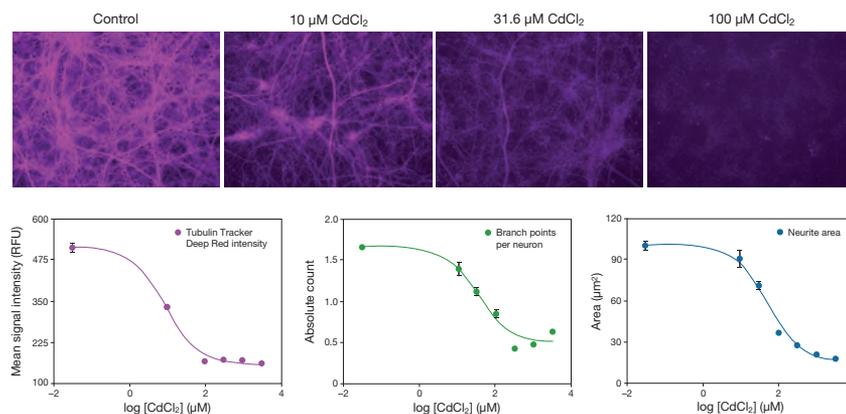


Figure 8. Pharmacological screening of neurite outgrowth with Tubulin Tracker Deep Red. Gibco™ Primary Rat Cortex Neurons (Cat. No. A1084001) were cultured for 14 days *in vitro* using the Gibco™ B-27™ Plus Neuronal Culture System (Cat. No. A3653401) and Gibco™ GlutaMAX™ Supplement (Cat. No. 35050061). Cells were then treated with different concentrations of cadmium chloride (CdCl₂) for 18 hr and stained using Invitrogen™ Tubulin Tracker™ Deep Red (Cat. No. T34077). Neurite morphology was assessed using the Thermo Scientific™ CellInsight™ CX5 High-Content Screening (HCS) Platform.

the advantages of long-wavelength excitation in live cells, as well as the low cytotoxicity of Tubulin Tracker Deep Red in primary neuronal cultures.

Neurite outgrowth assays for pharmacological screening

The benefits of microtubule specificity and absence of cytotoxicity in live primary neuronal cultures prompted us to investigate the use of Tubulin Tracker Deep Red as a novel tool for pharmacological screening in neurons. Figure 8 shows a dose-response study in live rat cortical neurons. Upon treatment with cadmium chloride (CdCl₂), we quantified the dose-dependent toxicity in primary neurons by measuring Tubulin Tracker Deep Red intensity branch points per neuron, and neurite area using image segmentation software found on the Thermo Scientific™ CellInsight™ CX5 High-Content Screening (HCS) Platform. The results mirrored the known cytotoxic profile of CdCl₂ in neuronal cultures, providing proof-of-concept that Tubulin Tracker Deep Red can be utilized for a readout of these stereotypical neuronal traits to aid in the screening of new drugs aimed at a variety of neuronal degenerative diseases, including PD.

3D models for determining neurosphere maturation

3D models have emerged as effective systems for bridging traditional monolayer cell-based assays and *in vivo* animal experiments, and their adoption is increasing in many different research areas. Gibco™ Neurobasal™ Medium combined with Tubulin Tracker Deep Red was used to establish 3D neurospheres, a useful approach for neuronal disease modeling. To demonstrate the specificity of Tubulin Tracker Deep Red for microtubules in neural stem cell (NSC)-derived preparations, the total neuronal content of 3D neurospheres cultured using different growth conditions was assessed by measuring the overall intensity of labeling with Tubulin Tracker Deep Red (Figure 1). When comparing traditional Neurobasal Medium to Neurobasal Plus Medium in the presence and absence of Gibco™ CultureOne™ Supplement, clear differences in microtubule content across the four growth conditions were observed (data not shown). We found →

that cultures in Neurobasal Plus Medium were far better at promoting neuronal growth for applications in 3D neuronal models.

Research tools for the study of PD and other neurological diseases

Although the number of genes and proteins implicated in PD continues to grow, a majority of them are proving to be a challenge to study given their low abundance and a lack of quality reagents to investigate their functions. Invitrogen antibodies are tested in relevant models and applications, and their specificity is confirmed using siRNA- and CRISPR-mediated gene silencing, relative expression, and cell treatment. Learn more about our expanded antibody validation methods at thermofisher.com/antibodyvalidation, and search our complete portfolio of primary and secondary antibodies for flow cytometry, immunofluorescence, western blotting, ELISAs, and other applications at thermofisher.com/antibodies.

Tubulin Tracker reagents have been used to visualize microtubules in a wide variety of live cell types, and Tubulin Tracker Deep Red has been demonstrated to label 3D neurospheres. Tubulin Tracker Deep Red can be multiplexed with common blue, green, orange, and near-IR fluorescent reagents and enables the study of microtubule dynamics with extended time-lapse imaging, including pharmacological effects on microtubule networks in live neuronal cultures. Learn more at thermofisher.com/tubulintracker. ■

*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.

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| Product | Quantity | Cat. No. |
|---|--|----------------------|
| Antibodies for Parkinson's disease research: see Tables 1, 2, and 3. | | |
| Cell culture tools | | |
| Primary Rat Cortex Neurons | 1 x 10 ⁶ cells 4 x 10 ⁶ cells | A1084001 A1084002 |
| Primary Mouse Hippocampal Neurons | 1 x 10 ⁶ cells | A15587 |
| Neurobasal™ Medium | 500 mL | 21103049 |
| B-27™ Plus Neuronal Culture System | 1 system | A3653401 |
| GlutaMAX™ Supplement | 100 mL 20 x 100 mL | 35050061 35050079 |
| CultureOne™ Supplement (100X) | 5 mL | A3320201 |
| Cell imaging tools | | |
| CellInsight™ CX5 High-Content Screening (HCS) Platform | 1 each | CX51110 |
| CellInsight™ CX7 LZR High-Content Analysis (HCA) Platform | 1 each | CX7A1110LZR |
| EVOS™ Light Cube, Cy [®] 5 | 1 each | AMEP4656 |
| EVOS™ M7000 Imaging System | 1 system | AMF7000 |
| Microtubule labeling tools | | |
| Tubulin Tracker™ Deep Red | 60 slides 300 slides | T34077 T34076 |
| Tubulin Tracker™ Green (Oregon Green™ 488 Taxol, Bis-Acetate) | 60 slides 300 slides | T34078 T34075 |
| Tubulin Tracker™ Variety Pack | 2 x 60 slides | T34079 |

Characterize immune cell identity and purity

eBioscience Essential Phenotyping antibody panels and CTS PureQuant qPCR-based assays.

One of the many challenges in immunology, cell therapy, and related fields is the accurate estimation of T cell identity and purity within heterogeneous immune cell populations due to the diverse cell types, rare subsets, source material variability, and lack of available validated techniques [1]. Currently, the primary means of characterizing heterogeneous cell populations is by flow cytometry [2]. Although there are commonly used antibody markers for immunophenotyping, few validated panels are commercially available. Other technologies used to assess immune cell populations include next-generation sequencing, ELISAs, qPCR assays, and array-based assays, but these methods are typically low-throughput, time-consuming, and costly.

Invitrogen™ eBioscience™ Essential Phenotyping Kits and Applied Biosystems™ CTS™ PureQuant™ Assays provide two different but complementary methods for characterizing immune cells. Each method allows researchers to characterize cytotoxic T (CD8⁺) cells, regulatory T (Treg) cells, and T helper 17 (Th17) cells. The eBioscience Essential Phenotyping Kits are antibody panels that enable the identification of a large number of markers and cell types using flow cytometry. The CTS PureQuant Assays are qPCR-based methylation assays that are rapid and potentially more accurate than antibody panels, depending on the workflow. Throughout the development of these methods, we compared their performance on the same cell populations, allowing us to see the benefits and drawbacks of each assay. In each case, these methods were designed to provide a flexible and efficient system for determining the identity and purity of immune cell subtypes. →

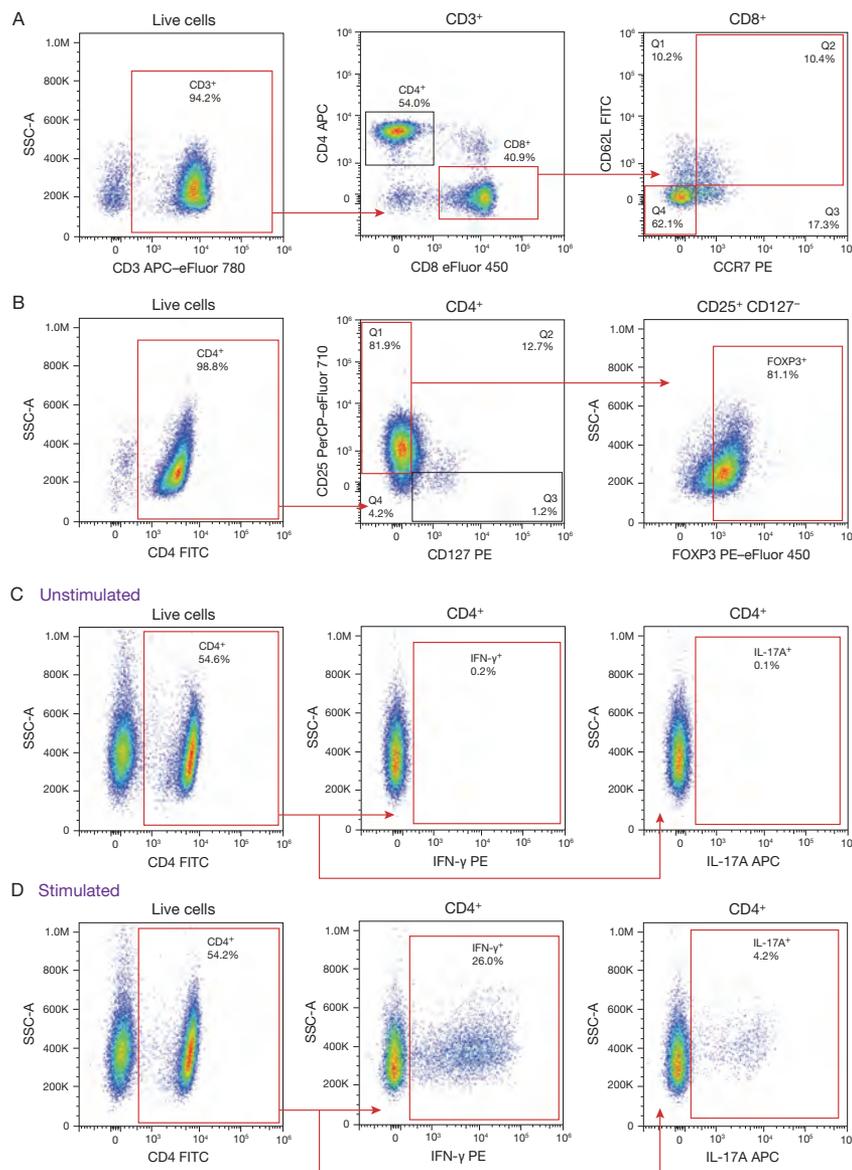


Figure 1. Gating strategy for three eBioscience Essential Phenotyping antibody panels. (A) Using the Invitrogen™ eBioscience™ Essential Human T Cell Phenotyping Kit (Cat. No. A42923), we identified CD3⁺ cells (pan T cells, left plot), followed by CD4⁺ cells (helper T cells) and CD8⁺ cells (cytotoxic T cells) (middle plot). CD8⁺ cells were further separated into naive cells (CD8⁺CCR7⁺CD62L⁻) and effector cells (CD8⁺CCR7⁻CD62L⁻) (right plot). **(B)** Using the Invitrogen™ eBioscience™ Essential Human Treg Phenotyping Kit (Cat. No. A42925), we identified CD4⁺ cells (left plot), followed by CD25⁺CD127⁻ cells (middle plot). Then, the definitive Treg marker FOXP3 was used to identify the Treg population (right plot). **(C, D)** Using the Invitrogen™ eBioscience™ Essential Human Th1/Th17 Phenotyping Kit (Cat. No. A42927), we identified CD4⁺ cells, followed by IFN-γ⁺ (Th1, middle plot) and IL-17A⁺ (Th17, right plot) cells. Before analysis, T cells were either **(C)** left unstimulated or **(D)** stimulated with PMA and ionomycin to elevate levels of IL-17A⁺ cells, and then treated with a protein transport inhibitor to prevent efflux. For all experiments, debris, doublets, and dead cells were gated out. All gates are set using fluorescence minus one (FMO) and isotype controls, and recommended gating methodology is explained in detail in the user manual for each kit. Cells were analyzed using the Invitrogen™ Attune™ NxT Flow Cytometer (blue/red/violet6/yellow, Cat. No. A29004).

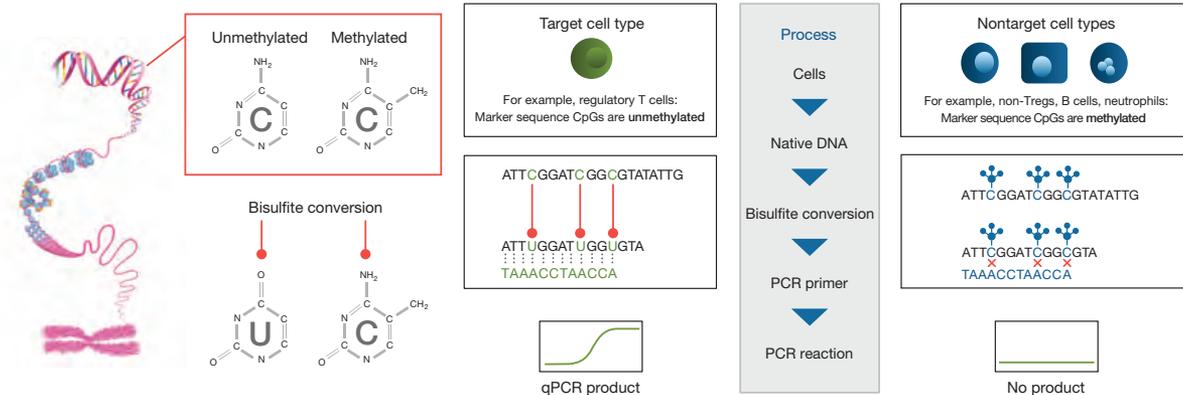


Figure 2. Methylation assay principle, showing the high-level process and the difference between methylated and unmethylated genomic DNA in target and nontarget cell types, respectively. The Applied Biosystems™ CTS™ PureQuant™ Assays use DNA methylation at specific sites that serve as unique identifiers of a target cell type.

eBioscience Essential Phenotyping antibody panels

Despite the fact that flow cytometry is a highly utilized characterization tool, many antibody panels lack validation and standardized analysis strategies. The eBioscience Essential Human T Cell, Treg, and Th1/Th17 Phenotyping Kits were designed for easy and consistent identity testing of T cells using flow cytometry instruments with a standard three-laser configuration. They include high-quality Invitrogen™ eBioscience™ antibodies and optional isotype controls, as well as a ready-to-use protocol for experimental setup, gating strategy, and analysis.

The three panels detect and quantify both cell-surface and intracellular markers for T cells, Treg cells, and Th1 and Th17 populations. After analysis using the appropriate gating strategy (Figure 1), each panel determines the percentage of multiple cell types within the population. The eBioscience Essential Human T cell panel includes antibodies that identify pan T cells (CD3⁺), helper T cells (CD4⁺), cytotoxic T cells (CD8⁺), and both naive T cells (CD8⁺CD62L⁺CCR7⁺) and effector T cells (CD8⁺CD62L⁻CCR7⁻). The eBioscience Essential Human Treg panel identifies Treg cells (CD4⁺CD25⁺CD127⁻FOXP3⁺). The eBioscience Essential Human Th1/Th17 panel identifies both Th1 cells (CD4⁺IFN-γ⁺) and Th17 cells (CD4⁺IL-17A⁺). These cell types play critical roles in the immune system. Effector cells will quickly kill tumor cells and die, while naive T cells will persist significantly longer. In contrast, Treg cells are important for peripheral immune tolerance and quell hyperactive inflammatory responses. Inflammatory immune cells such as Th17 cells are responsible for recruiting neutrophils and macrophages to protect against extracellular bacterial infection; however, Th17 cells also play a major role in autoimmune disorders. Ultimately, these panels provide a validated turnkey method for researchers to identify and monitor T cell subtypes.

CTS PureQuant methylation assays

The CTS PureQuant CD8⁺ T Cell, Treg, and Th17 Assays provide another highly standardized method for determining T cell subtypes that offers flexibility with regard to both workflow and source material (fresh or frozen cells, or isolated genomic DNA). These assays utilize cell type-specific genomic DNA methylation patterns to identify and quantify specific immune cell subtypes in a heterogeneous cell population using a qPCR-based method. In this assay (Figure 2), ammonium bisulfite converts unmethylated cytosine to uracil indiscriminately throughout the entire genome. Primer pairs are specifically designed to anneal within target genes and the newly converted uracil bases. A sequence with methylated cytosine will not bind these primers and consequently will not be amplified during the qPCR reaction. All necessary controls such as calibrator (to correct for variation in bisulfite conversion efficiency), reference (to check assay performance), and standards are provided to create standard curves to derive copy number, which is normalized to total copy number (GAPDH) to determine the percentage of the cell type of interest.

Side-by-side comparisons

In order to evaluate these two methods, we tested three different donor samples using the eBioscience Essential Human T Cell Phenotyping Kit and the CTS PureQuant CD8⁺ T Cell Assay. When compared side by side, the two methods yielded consistent results for the measurement of the CD8⁺ population (Figure 3). For this characterization of donor samples, the antibody panel workflow required less time because all antibody targets are surface-bound receptors and therefore the cells do not require fixation or multiple rounds of staining.

Next, we characterized three different samples of purified Treg cells using both the eBioscience Essential Human Treg Phenotyping Kit and the CTS PureQuant Treg Assay (Figure 4). Purified Treg cells were used because these cells are in very low abundance, even within pan T cell populations. Once again, results were very consistent between methods. Because the eBioscience Essential Phenotyping antibody panels stain for the intracellular marker FOXP3, which requires cell fixation and permeabilization, the length of each workflow was comparable.

Lastly, we compared the eBioscience Essential Human Th1/Th17 Phenotyping Kit and the CTS PureQuant Th17 Assay. To identify Th17 cells in a T cell population using an IL-17A antibody and flow cytometry, cells first need to be stimulated with PMA and ionomycin to increase the normally low expression of IL-17A, the marker used to identify Th17 cells. Additionally, a protein transport inhibitor is required to prevent secretion of IL-17A by Th17 cells. As a control, cells were treated with the protein transport inhibitor but not with PMA and ionomycin. The qPCR-based methylation assay does not require this stimulation step, but for this comparison, the control and stimulated T cells were used with both the phenotyping panel (see gating strategy in Figures 1C and 1D) and the qPCR-based methylation assay. As seen in Figure 5, results based on methylation signatures are much higher and closer to the expected result than those achieved with the antibody panel. It is possible that the artificial stimulation needed for the antibody-based flow cytometry assay is not an optimally effective method for detecting transient analytes, at least when compared with detection of genomic methylation signatures. In addition to the extended time needed for the stimulation workflow, the eBioscience Essential Phenotyping antibody panels require live cells for the flow cytometry analysis, whereas the CTS PureQuant assays can be used with fresh or frozen cells or with isolated genomic DNA.

Learn more about immune cell characterization

The eBioscience Essential Phenotyping Kits and the CTS PureQuant Assays provide orthogonal methods to measure the identity and purity of heterogeneous immune cell populations. Whereas the phenotyping panels allow quantitation of more cell types and provide a faster workflow for surface-marker identification, the qPCR-based methylation assays offer an improvement in the workflow and accuracy with regard to Th17 identification. Both methods have significant benefits and, when used together, provide a robust means for determining the identity and purity of mixed immune cell populations. For more information, go to thermofisher.com/immunepanelkit and thermofisher.com/purequant. ■

| Product | Quantity | Cat. No. |
|---|------------------------|--------------------------|
| CTS™ PureQuant™ CD8+ T Cell Assay | 28 reactions | A43674 |
| CTS™ PureQuant™ Th17 Assay | 28 reactions | A43676 |
| CTS™ PureQuant™ Treg Assay | 28 reactions | A43675 |
| eBioscience™ Essential Human T Cell Phenotyping Kit | 100 reactions | A42923 |
| eBioscience™ Essential Human Th1/Th17 Phenotyping Kit | 100 reactions | A42927 |
| eBioscience™ Essential Human Treg Phenotyping Kit | 100 reactions | A42925 |
| eBioscience™ Fixable Viability Dye, eFluor™ 506 | 100 tests 500 tests | 65-0866-14 65-0866-18 |
| eBioscience™ Flow Cytometry Staining Buffer | 200 mL 600 mL | 00-4222-57 00-4222-26 |

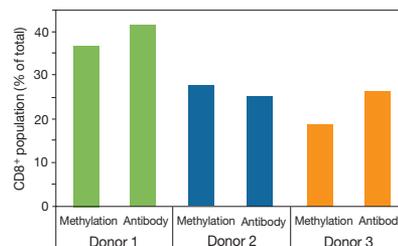


Figure 3. CD8+ population analysis. T cells isolated from three different donors were analyzed using both the Applied Biosystems™ CTS™ PureQuant™ CD8+ T Cell Assay (methylation, Cat. No. A43674) and the Invitrogen™ eBioscience™ Essential Human T Cell Phenotyping Kit (antibody, Cat. No. A42923).

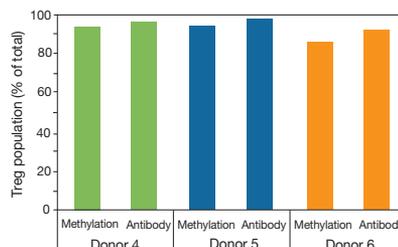


Figure 4. Treg population analysis. Purified Treg cells from three different donors were analyzed using the Applied Biosystems™ CTS™ PureQuant™ Treg Assay (methylation, Cat. No. A43675) and the Invitrogen™ eBioscience™ Essential Human Treg Phenotyping Kit (antibody, Cat. No. A42925).

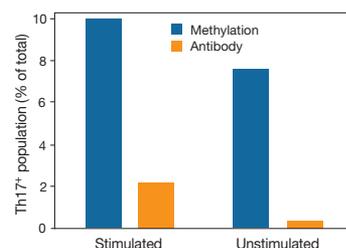


Figure 5. Th17 population analysis. Stimulated and unstimulated T cells from a single donor were analyzed using the Applied Biosystems™ CTS™ PureQuant™ Th17 Assay (methylation, Cat. No. A43676) and the Invitrogen™ eBioscience™ Essential Human Th1/Th17 Phenotyping Kit (antibody, Cat. No. A42927).

References

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Enhance immunodetection in neurons and other complex samples

Development and optimization of Alexa Fluor Plus secondary antibodies.

Obtaining the best possible results in fluorescence-based cell imaging requires that all reagents used in the workflow are optimized to maximize signal over background. Optimization is especially critical when working with challenging experimental systems, samples that are hard to obtain like neurons, or low-abundance cellular targets that require signal amplification. An essential yet often overlooked factor in the workflow is the selection and optimal use of the fluorescent secondary antibodies. Invitrogen™ Alexa Fluor™ Plus secondary antibodies were specifically developed to provide superior sensitivity, a high signal-to-noise (S/N) ratio, and low cross-reactivity for the detection of low-abundance proteins in critical samples and experiments. This article describes how we developed these antibodies using a high-throughput workflow that allowed us to perform complex, multivariable analyses on the different antibody candidates under a range of experimental conditions, resulting in the selection of the best possible Alexa Fluor Plus antibody conjugates.

Overview of the high-throughput workflow

Testing multiple dyes and dye conjugates across a range of samples and dilutions requires a high-throughput solution that enables reliable quantitation, reproducibility, scalability, and analysis of hundreds of individual data points. To accomplish this task, we utilized the Thermo Scientific™ CellInsight™ CX5 High-Content Screening (HCS) Platform

equipped with Thermo Scientific™ HCS Studio™ Cell Analysis Software and semi-automated sample handling. Cells were seeded, fixed, and permeabilized in 96-well or 384-well plates, with washing and processing performed on a Thermo Scientific™ Multidrop™ Combi Reagent Dispenser. Multiwell replicates of cells were incubated in the presence (signal) or absence (background) of primary antibody, followed by secondary antibody incubation, with matched conditions for washing, nuclear staining, and signal acquisition across all wells.

Finding the optimal working concentration for potential Alexa Fluor Plus candidates

The first step of the testing process was focused on determining the optimal working concentration for all of the Alexa Fluor Plus secondary antibody candidates. In the example shown in Figure 1, the fluorescence intensity of goat anti-mouse IgG secondary antibodies was measured with or without primary antibody at concentrations spanning 0.1 ng/mL to >10 µg/mL (Figure 1A–C). Interestingly, background fluorescence in the secondary antibody control (samples without primary antibody) increased drastically at concentrations over 10 µg/mL (Figure 1B), resulting in suboptimal S/N ratios at these higher secondary antibody concentrations (Figure 1C). It is important to note that the optimal secondary antibody concentration can vary with the label and species specificity of the secondary antibody, as

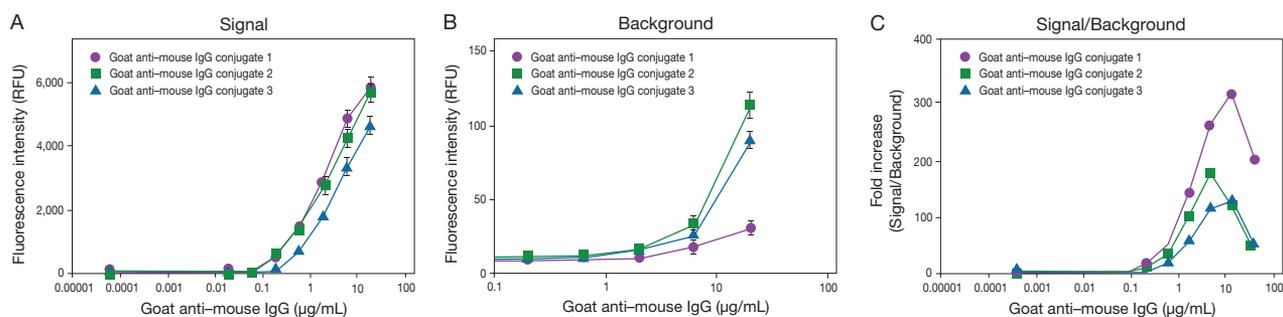


Figure 1. Titration of fluorescent secondary antibodies for optimized signal-to-noise (S/N) ratio. Fluorescent secondary antibodies—including Invitrogen™ Alexa Fluor™ Plus development candidates—were titrated to determine the optimal concentration range for maximal S/N ratio. Fluorescence intensities are shown as relative fluorescence units (RFU) using (A) secondary antibody in the presence of a corresponding primary antibody to determine signal or (B) secondary antibody in the absence of a primary antibody, to control for nonspecific background noise due to the secondary antibody. (C) The S/N values for data in panels A and B are shown. The optimal S/N ratio for all secondary antibody candidates is obtained using 1–10 µg/mL and declines with higher concentrations of secondary antibody. Data were acquired on a Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform equipped with Thermo Scientific™ HCS Studio™ Cell Analysis Software.

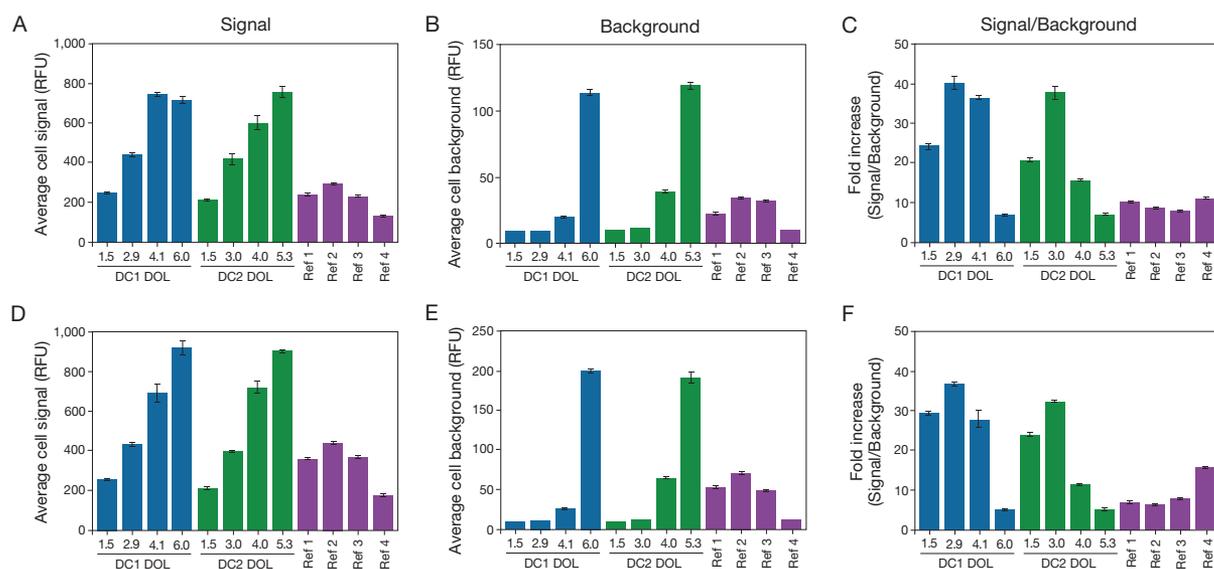


Figure 2. Using a guard banding approach to determine the optimal degree of labeling (DOL) of Alexa Fluor Plus secondary antibodies. DOL guard banding of two Invitrogen™ Alexa Fluor™ Plus development candidates (for the Alexa Fluor Plus 594 goat anti–mouse IgG secondary antibody) and benchmark secondary antibodies in (A–C) cytoplasmic and (D–E) nuclear cell staining applications. (A, D) Signal and (B, E) background are shown as average cell intensities (in RFU); (C, F) signal-to-noise (S/N) ratios are expressed as fold increase of the signal over background. All determinations were generated with RFU data collected from 200 cells per well, n = 3 wells per condition tested. Data were acquired on a Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform equipped with Thermo Scientific™ HCS Studio™ Cell Analysis Software.

well as with the primary antibody. Therefore, we always recommend that the secondary antibody be titrated for each primary antibody used in an imaging experiment to help ensure that optimal S/N ratios are obtained. Omitting this titration could result in a decreased range of detection and a lower S/N ratio, which can translate into loss of signal specificity or increased background.

Screening Alexa Fluor Plus candidates with different DOLs for the optimal S/N ratios

To determine the optimal degree of labeling (DOL) of Alexa Fluor Plus conjugates in development, we used highly cross-adsorbed IgGs that were labeled and purified with a range of DOLs using an approach called guard banding, which defines the acceptable offset from a given specification (in this case, the DOL that produces the optimal S/N). These conjugates were then used for a series of determinations of signal intensity and background fluorescence at various concentrations in cells that had been incubated with or without primary antibody.

Figure 2 shows an example of DOL guard banding of two development candidates (DC1 and DC2) that were tested in parallel at 2 µg/mL to determine 1) optimal DOL range and 2) which candidate to use for the Alexa Fluor Plus 594 goat anti–mouse IgG secondary antibody, based on S/N performance. In this example, the secondary antibodies

were used to detect an anti-tubulin primary antibody in the cytoplasm of A549 cells (Figure 2A–C). A no–primary antibody control was used to detect background in the same area of the cytoplasm. DC1 and DC2 were prepared with DOLs between 1.5 and 6.2 and benchmarked to spectrally similar red-fluorescent goat anti–mouse IgG secondary antibodies from Thermo Fisher Scientific and other suppliers at matched concentrations (Figure 2, purple bars). As expected, as the average number of fluorophores per antibody molecule increased, the signal intensity (measured in relative fluorescence or RFU) for DC1 (blue bars) and DC2 (green bars) also increased, from an average of 200 RFU for a DOL of 1–2 to over 700 RFU for a DOL of 4–6 (Figure 2A). However, increasing the DOL can also lead to an increase in background, as shown in this example for the samples with high DOL values of 5–6 (Figure 2B). Ultimately, the S/N ratio was used as the critical performance indicator when evaluating the development candidates (Figure 2C). Both development candidates showed higher S/N ratios over benchmark secondary antibodies; however, DC1 showed a higher S/N ratio over a broader range of DOLs (Figure 2C).

To confirm these results using a nuclear target, we performed the same guard banding approach by probing A549 cells for etoposide-induced DNA damage and subsequent phosphorylation of the histone H2AX (pH2AX) using an anti-pH2AX primary antibody. Next we →

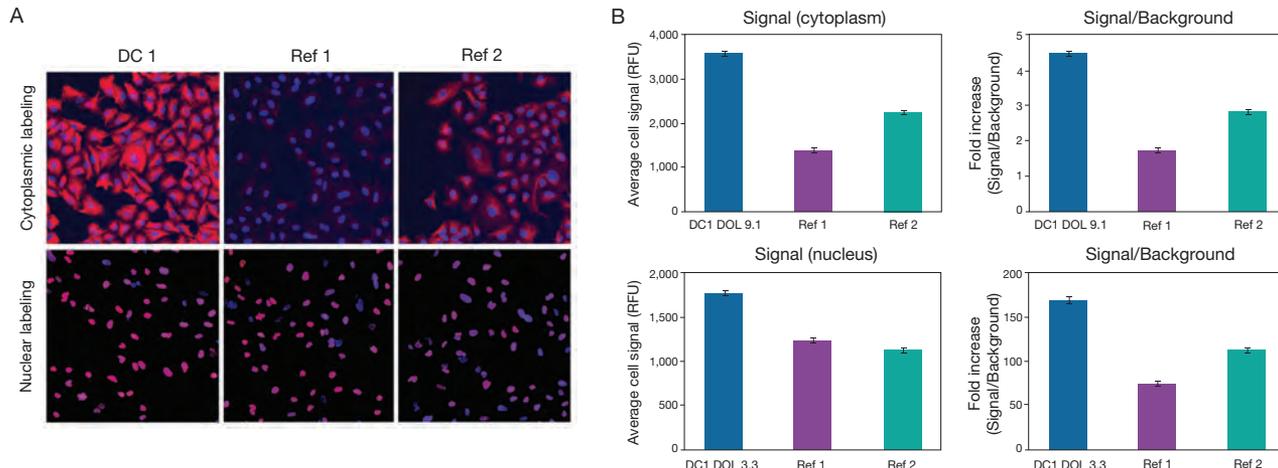


Figure 3. Comparison of cytoplasmic and nuclear target signal-to-noise (S/N) ratios using Alexa Fluor Plus antibody conjugates. (A) Top row: Representative pseudocolored images show the differences in signal intensity and S/N ratios after staining with Invitrogen™ Alexa Fluor™ Plus 555 donkey anti-mouse IgG antibody (Cat. No. A32773) and benchmark secondary antibody (Ref 1 and Ref 2, above) for the detection of tubulin in the cytoplasm (red) of A549 cells, counterstained with DAPI (blue). Bottom row: Alexa Fluor Plus 594 goat anti-rabbit IgG antibody (Cat. No. A32740) and benchmark secondary antibody (Ref 1 and Ref 2, above) were used for the detection of pH2AX in the nuclei of etoposide-treated A549 cells (purple) counterstained with DAPI (blue). (B) Quantitation of average signal intensity and S/N values are also shown.

measured the average signal intensity and background fluorescence (with and without primary antibody) in cell nuclei (Figure 2D–E). Cells were labeled with the same two secondary antibody candidates using the same DOL range as in Figure 2A–C, and the S/N ratios were analyzed. As shown in Figure 2D–E, the results were very consistent between cytoplasmic and nuclear staining, confirming that both development candidates showed higher S/N ratios over the benchmark secondary antibodies and that DC1 showed higher S/N ratios over a broader range of DOLs for this nuclear target. Based on these results, DC1 was selected for the new Alexa Fluor Plus 594 goat anti-mouse IgG secondary antibody with a DOL range of 2–4.

Table 1 provides an overview of two of the six species-specific Alexa Fluor Plus secondary antibodies (donkey anti-mouse IgG and donkey anti-rabbit IgG), with DOL ranges, excitation/emission maxima, and tested applications; Figure 3 shows representative pseudocolored images. It is important to note that, depending on the abundance of the target, performance of the primary antibody, and complexity of the cell system, Alexa Fluor Plus secondary antibodies enable a significantly more robust detection of target proteins in most cases, primarily by boosting signal intensity, decreasing background levels, or both.

Testing Alexa Fluor Plus antibodies in neuronal systems

Alexa Fluor Plus secondary antibodies were used in a variety of cell systems. Two recent examples are shown in Figures 4 and 5 and

in several published articles [1-4]. In Figure 4, Alexa Fluor Plus 488 donkey anti-mouse IgG and Alexa Fluor Plus 555 donkey anti-rabbit IgG secondary antibodies (Figure 4A) were used for the detection of neuronal markers HuC/D and MAP2 in rat cortical neurons (Figure 4A), and compared with results obtained with the benchmark secondary antibodies (Figure 4B). This comparison demonstrates that the Alexa Fluor Plus secondary antibodies, optimized during development using the CellInsight CX5 HCS Platform, deliver the expected superior performance over benchmark secondary antibodies, providing higher S/N

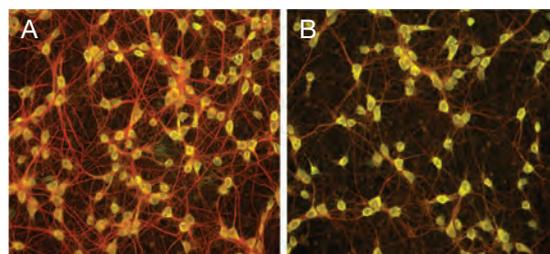


Figure 4. Enhanced detection of neuronal detail using Alexa Fluor Plus antibody conjugates. Invitrogen™ Alexa Fluor™ Plus 555 donkey anti-rabbit IgG (Cat. No. A32794) and Invitrogen™ Alexa Fluor™ Plus 488 donkey anti-mouse IgG (Cat. No. A32766) secondary antibodies were used to detect anti-MAP2 (red) and anti-HuC/D (green/yellow, as a result of overlay with red MAP2 labeling) primary antibodies in rat cortical neurons. Contrast-matched images show comparison of staining with (A) Alexa Fluor Plus vs. (B) benchmark secondary antibodies. Data were acquired on a Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform equipped with Thermo Scientific™ HCS Studio™ Cell Analysis Software.

ratios and greater image detail in fluorescence microscopy applications. In Figure 5, Alexa Fluor Plus 555 donkey anti-mouse IgG and Alexa Fluor Plus 647 donkey anti-rabbit IgG secondary antibodies used in a cardiovascular lateral flow experimental system for detection of membrane-associated proteins improved the sensitivity of target detection over that obtained using benchmark secondary antibodies (Figure 5B) in the same system under matched conditions.

Use Alexa Fluor Plus secondary antibodies for your experiments

For the detection of low-abundance targets in immunofluorescence imaging, there are multiple options available to researchers, each with its unique set of pros and cons. Depending on the experimental system or the level of signal amplification needed, the use of biotinylated secondary antibodies in conjunction with dye-labeled streptavidin conjugates or tyramide-based signal amplification may be considered. However, due to its simplicity and consistency, the most common signal amplification technique is the use of fluorescent secondary antibodies.

Alexa Fluor Plus secondary antibodies were developed to deliver more sensitive fluorescence detection of primary antibodies with greater S/N ratios, enabling more robust detection of low-abundance targets in hard-to-obtain samples and critical experiments. The Alexa Fluor Plus secondary antibodies, available in a wide range of fluorescent colors and species specificity (Table 2), have been widely referenced in scientific publications and used in a great variety of cell model systems and applications. To learn more and find a complete list of available Alexa Fluor Plus secondary antibodies, go to thermofisher.com/alexafuorplus. ■

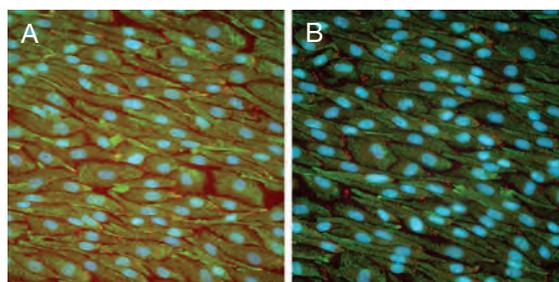


Figure 5. Improved sensitivity and specific signal brightness with Alexa Fluor Plus antibody conjugates. A flow-aligned human aortic endothelial cell (HAEC) monolayer stained for plasma membrane-associated proteins using (A) Invitrogen™ Alexa Fluor™ Plus 555 donkey anti-mouse IgG (green, Cat. No. A32773) and Invitrogen™ Alexa Fluor™ Plus 647 donkey anti-rabbit IgG (red, Cat. No. A32795) or (B) traditional Alexa Fluor™ secondary antibodies under matched conditions. Data used with permission from Julia J. Mack and Julianne W. Ashby, Department of Medicine/Cardiology, University of California Los Angeles.

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Table 1. DOL ranges for Alexa Fluor Plus donkey anti-mouse IgG and donkey anti-rabbit IgG secondary antibodies and their applications.

| | Conjugate | DOL range | Ex/Em max (nm) | Applications (tested, published) | Cat. No. |
|--------------------|----------------------|-----------|----------------|----------------------------------|----------|
| Donkey anti-mouse | Alexa Fluor Plus 488 | 6-10 | 490/525 | IF/ICC [1,2], WB | A32766 |
| | Alexa Fluor Plus 555 | 8-11 | 555/580 | IF/ICC [3], WB | A32773 |
| | Alexa Fluor Plus 594 | 2-4 | 590/617 | IF/ICC | A32744 |
| | Alexa Fluor Plus 647 | 5-8 | 650/665 | IF/ICC, WB | A32787 |
| | Alexa Fluor Plus 680 | 5-8 | 679/702 | IF/ICC, WB | A32788 |
| | Alexa Fluor Plus 800 | 2-5 | 777/794 | IF/ICC, WB | A32789 |
| Donkey anti-rabbit | Alexa Fluor Plus 488 | 5-8 | 490/525 | IF/ICC, WB | A32790 |
| | Alexa Fluor Plus 555 | 7-10 | 555/580 | IF/ICC, WB | A32794 |
| | Alexa Fluor Plus 594 | 2-3.5 | 590/617 | IF/ICC [2] | A32754 |
| | Alexa Fluor Plus 647 | 4-7 | 650/665 | IF/ICC [4], WB | A32795 |
| | Alexa Fluor Plus 680 | 4-6 | 679/702 | IF/ICC, WB | A32802 |
| | Alexa Fluor Plus 800 | 2-4 | 777/794 | IF/ICC, WB | A32808 |

Table 2. Alexa Fluor Plus secondary antibody catalog numbers, by species specificity and fluorescent color.

| Secondary antibody | Alexa Fluor Plus 488 | Alexa Fluor Plus 555 | Alexa Fluor Plus 594 | Alexa Fluor Plus 647 | Alexa Fluor Plus 680 | Alexa Fluor Plus 800 |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Goat anti-rabbit IgG | A32731 | A32732 | A32740 | A32733 | A32734 | A32735 |
| Goat anti-mouse IgG | A32723 | A32727 | A32742 | A32728 | A32729 | A32730 |
| Goat anti-chicken IgY | A32931 | A32932 | A32759 | A32933 | A32934 | A32935 |
| Donkey anti-goat IgG | A32814 | A32816 | A32758 | A32849 | A32860 | A32930 |
| Donkey anti-rabbit IgG | A32790 | A32794 | A32754 | A32795 | A32802 | A32808 |
| Donkey anti-mouse IgG | A32766 | A32773 | A32744 | A32787 | A32788 | A32789 |

Validated RBP antibodies: Enliven your RIP protocols

RNA-binding protein antibodies validated for RNA immunoprecipitation.

RNA-binding proteins (RBPs) recognize specific RNA sequences or structures and control many molecular functions, including transcription, splicing, 5' RNA capping, polyadenylation, mRNA export, ribosome assembly, translation, and RNA decay. These diverse functions allow RBPs to wield exquisite control over gene expression that is either dependent on or independent of transcription. Because these proteins play significant roles in regulating gene expression, identifying which RNAs a specific RBP associates with can provide powerful information for dissecting downstream functions. Similar to chromatin immunoprecipitation (ChIP), RNA immunoprecipitation (RIP) often starts with the crosslinking of live cells, followed by immunoprecipitation of RNA–protein complexes using a specific RBP antibody, and recovery and identification of the RNA sequences [1]. Thermo Fisher Scientific has instituted validation* programs for Invitrogen™ RBP antibodies that use RIP and RIP-western (RIP followed by western blotting to determine the protein immunoprecipitated). Here we highlight a few targets in our rapidly growing RBP antibody portfolio.

RNA binding by the PRC2 complex

The polycomb repressive complex 2 (PRC2), which has histone methyltransferase activity, plays a critical role in gene regulation by controlling the trimethylation of histone H3 on lysine 27 (H3K27me3)—one of the hallmarks of transcriptionally silent chromatin. Dysregulation of PRC2 has been implicated in several types of cancer, and PRC2 subunits have long been targets for therapeutics. Although PRC2 subunits have been shown to bind to numerous types of RNA, the complex's function is still rather elusive [2]. For example, PRC2 interacts with many nascent RNAs, leading researchers to question the function of this promiscuous binding.

The “junk-mail model” suggests that promiscuous binding of PRC2 serves as a checkpoint—PRC2 identifies target genes that have escaped repression by binding to the newly transcribed RNA and then scanning the local chromatin for H3K27me3, which signals that the gene should be silenced. Alternatively, the “antagonistic model” is based on competition of RNA and chromatin for the binding of PRC2. For highly expressed genes, PRC2 binding shifts to the nascent RNA; for transcriptionally silent genes, RNA levels are low so PRC2 binding shifts to chromatin. Furthermore, it has been proposed that PRC2 may prefer certain RNA secondary structures—such as RNA G-quadruplexes [3]. The ability to analyze which RNAs bind the PRC2 complex is essential for a better understanding of the biological consequences of these interactions. Figure 1 shows that SUZ12, a core component of PRC2, can be immunoprecipitated using a recombinant monoclonal SUZ12 antibody and then analyzed by RIP-western. RIP and other experiments that examine RNA–protein interactions will help to clarify the function of PRC2 binding to RNA.

mRNA binding by IMPs

The highly conserved IMP family of mRNA-binding proteins, also known as insulin-like growth factor 2 mRNA binding proteins (IGF2BPs), regulate RNA localization, translation, and stability, and associate with over 1,000 target mRNAs [4]. IMP1 (IGF2BP1) and IMP3 (IGF2BP3) are primarily

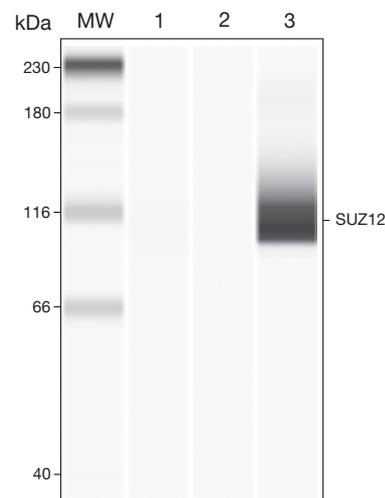


Figure 1. Immunoprecipitation of SUZ12 in K562 cells. Antigen–antibody complexes were formed by incubating ~500 µg whole cell lysate with 5–10 µL Invitrogen™ SUZ12 Recombinant Rabbit Monoclonal Antibody (clone 7H26L21, Cat. No. 702490) and rotating for 60 min at room temperature. The immune complexes were captured on 625 µg Invitrogen™ Dynabeads™ M-280 Sheep Anti-Rabbit IgG super-paramagnetic beads (Cat. No. 11203D) and washed extensively. They were then eluted and analyzed on the ProteinSimple™ Simple Western™ system (Bio-Techne) with the same antibody used for immunoprecipitation (IP) at a dilution of 1:25, followed by a 1:100 dilution of secondary antibody: lane 1, the input; lane 2, no antibody used for IP; lane 3, target-specific antibody used for IP. Data courtesy of the GW Yeo lab, University of California San Diego, as part of the ENCODE project.

expressed during embryogenesis, although re-expressed in various tumor tissues [5,6]. IMP2 (IGFBP2) has been observed during development and in adult mice and has been shown to be expressed at elevated levels in cancer cells [7]. Although their roles are still being investigated, the IMPs selectively bind to and regulate their RNA targets. Dysregulated binding due to aberrant expression is associated with different types of cancer.

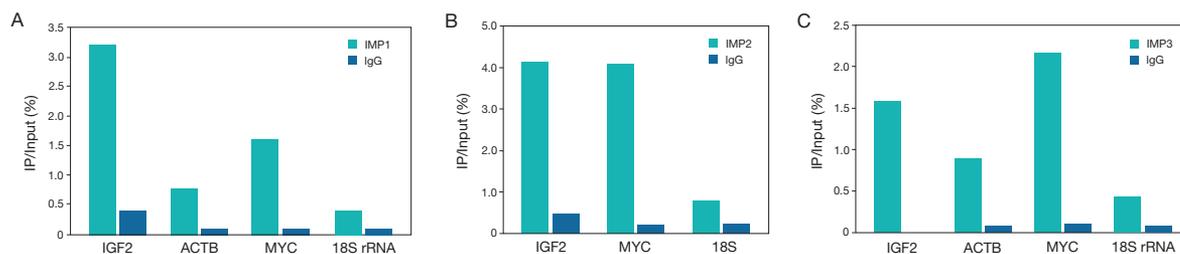


Figure 2. Detection of IMP binding to specific RNAs using RIP. The binding of endogenous IMPs to specific RNAs was determined by RNA immunoprecipitation (RIP) using Invitrogen™ recombinant polyclonal antibodies to (A) IMP1 (IGF2BP1) (Cat. No. 712138), (B) IMP2 (IGF2BP2) (Cat. No. 712137), and (C) IMP3 (IGF2BP3) (Cat. No. 712139) on clarified whole cell lysate from 2 x 10⁶ HepG2 cells. In all experiments, normal rabbit IgG was used as a negative IP control. Immunoprecipitated RNA was purified using the Invitrogen™ RiboPure™ RNA Purification Kit (Cat. No. AM1924) and analyzed by RT-qPCR using the Applied Biosystems™ Power SYBR™ Green RNA-to-C_T™ 1-Step Kit (Cat. No. 4389986) with primer pairs for IGF2, MYC, and ACTB mRNA (positive), and 18S rRNA (negative). Data are presented as the fraction of RNA immunoprecipitated (IP) normalized to the total amount of RNA used for immunoprecipitation (input).

Several target mRNAs were used with RIP to explore the specificity of recombinant polyclonal IMP antibodies in HepG2 cells (Figure 2), a human liver cancer cell line. The IGF2 transcript is a target for all three IMPs, although IMP1 inhibits translation, whereas IMP2 and IMP3 enhance translation. ACTB and MYC transcripts have been described as targets for IMP1, but enrichment of these mRNAs was seen for all three IMPs in HepG2 cells, which may be related to the role of IMP2 and IMP3 in liver cancer. IMPs have not been known to associate with 18S rRNA, and our RIP data are consistent with this observation.

Techniques for examining RNA–protein interactions

RIP is an excellent technique for identifying RNAs within an RNA–protein complex but may not be well suited for identifying direct RNA–protein interactions. A modification of RIP—the crosslinking and immunoprecipitation technique (CLIP)—uses UV crosslinking and stringent conditions to isolate only the specific RBP of interest and RNA species to which it is bound, and not other proteins and RNA contained within the complex. Several refinements have been developed, including the enhanced CLIP technique (eCLIP) shown in Figure 3 [8]. Immunoprecipitation with eIF4E and hnRNP K monoclonal antibodies demonstrate that they bind at different locations in TRA2A, as shown by shifts in peak density.

Find RIP-validated antibodies for your research

RIP, CLIP, and other immunoprecipitation methods will be essential for mapping the binding sites of RBPs across the transcriptome. Find specific, RIP-validated antibodies at thermofisher.com/antibodies. ■

*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.

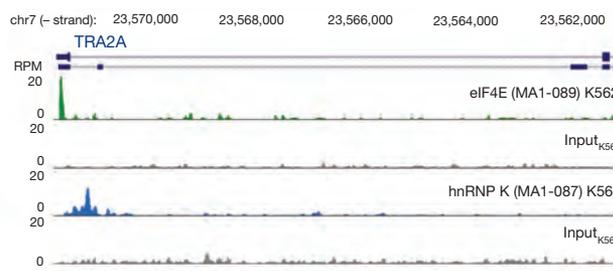


Figure 3. eIF4E and hnRNP K read density in reads per million (RPM). Shown are eCLIP with input controls in K562 cells at TRA2A. Invitrogen™ eIF4E Monoclonal Antibody (clone 5D11, Cat. No. MA1-089) and Invitrogen™ hnRNP K Monoclonal Antibody (clone F45 P9 C7, Cat. No. MA1-087) were used for the immunoprecipitation (see experimental details in [8]). Data courtesy of the GW Yeo lab, University of California San Diego, as part of the ENCODE project.

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| Product | Quantity | Cat. No. |
|--|---------------|----------|
| Dynabeads™ M-280 Sheep Anti-Rabbit IgG | 2 mL | 11203D |
| eIF4E Monoclonal Antibody (clone 5D11) | 100 µg | MA1-089 |
| hnRNP K Monoclonal Antibody (clone F45 P9 C7) | 100 µg | MA1-087 |
| IGF2BP1 Recombinant Polyclonal Antibody (clone 18HCLC) | 100 µg | 712138 |
| IGF2BP2 Recombinant Polyclonal Antibody (clone 1HCLC) | 100 µg | 712137 |
| IGF2BP3 Recombinant Polyclonal Antibody (clone 6HCLC) | 100 µg | 712139 |
| Power SYBR™ Green RNA-to-C _T ™ 1-Step Kit | 100 reactions | 4389986 |
| RiboPure™ RNA Purification Kit | 50 preps | AM1924 |
| SUZ12 Recombinant Rabbit Monoclonal Antibody (clone 7H26L21) | 100 µg | 702490 |

Investigate neuronal cellular senescence in age-related diseases

CellEvent Senescence Green Probe for fluorescence imaging and flow cytometry.

Cell biologists are focusing on the causes and treatments of age-related diseases, given increased life expectancies and an aging human population. Cellular senescence—exhibited by cells that have stopped dividing but remain metabolically active—is an important pathway for controlling unlimited cell division. However, if senescent cells are not removed by the immune system, a chronic pro-inflammatory environment ensues that can affect nearby healthy cells and increase the risk of age-related diseases. Senescent cells are more prevalent with increased age and are thought to play a role in many age-related pathologies, including neurodegenerative diseases such as Parkinson's and Alzheimer's as well as inflammatory diseases such as osteoarthritis and cardiovascular disease [1].

Recent studies in mouse models have shown that specific targeting of senescent cells with a senolytic cocktail produced longer healthspans and lifespans [2]. The development of senolytic drugs, which can selectively kill senescent cells or inhibit cellular senescence, is dependent on characterization of the senescence pathway and its signaling molecules, as well as on development of research tools for identifying senescent cells in various tissues and biological samples.

Multifactor detection of senescence

Senescent cells are characterized by the release of pro-inflammatory cytokines and chemokines, increased beta-galactosidase activity (senescence-associated (SA) β -Gal), heterochromatin foci (SAHF), and changes in cellular morphology [3]. Because there is no single marker of senescence, the identification

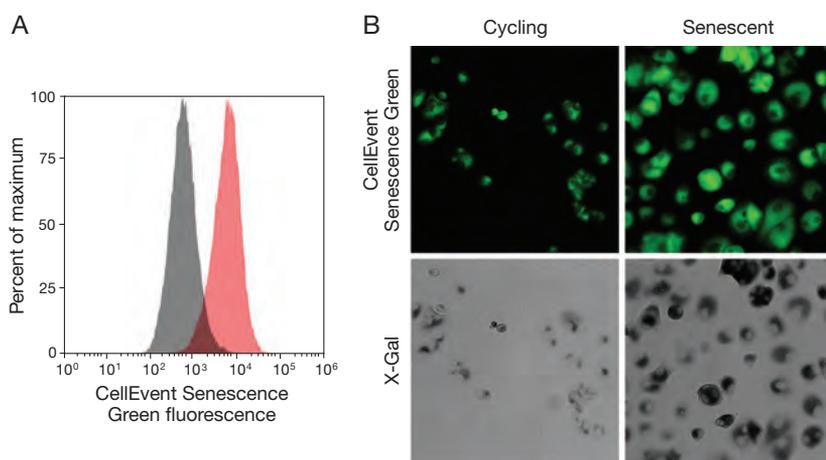


Figure 1. Detection of senescent cells with CellEvent Senescence Green Probe by flow cytometry and fluorescence imaging. T47D cells were treated with 5 μ M palbociclib for 16 days or left untreated. After treatment, cells were fixed with 4% formaldehyde for 10 min at room temperature, followed by senescence staining for 90 min in a 37°C incubator with no CO₂. **(A)** Flow cytometric analysis was performed using the Invitrogen™ CellEvent™ Senescence Green Flow Cytometry Assay Kit (Cat. No. C10840) on the Invitrogen™ Attune™ NxT Flow Cytometer, with 488 nm excitation and a 530/30 nm emission filter. The histogram shows clear separation of treated senescent cells (pink) from untreated cycling cells (gray). **(B)** Imaging analysis was performed using the Invitrogen™ CellEvent™ Senescence Green Detection Kit (Cat. No. C10850) on the Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform with the standard FITC/Alexa Fluor™ 488 settings. After fluorescence imaging, the same cells were stained overnight with X-Gal and imaged using brightfield, confirming that senescent cells with bright CellEvent Senescence Green staining are also labeled with X-Gal.

of senescent cells requires correlating the presence of multiple biomarkers, including proteins involved in cell cycle arrest (e.g., p16 and p21), proteins associated with DNA damage (e.g., p53 and p21), and increased β -Gal expression.

The detection of senescence based on the upregulation of β -Gal can be accomplished using a β -Gal substrate such as X-Gal (5-bromo-4-chloro-3-indolyl β -D-galactopyranoside), a colorimetric substrate that produces a blue-green precipitate upon enzymatic cleavage. Since the mid-1990s, X-Gal has been considered the gold standard for labeling senescent cells in tissue and biological samples [4]; yet, the X-Gal assay has several drawbacks, including its long incubation time and the difficulty of combining it with other staining protocols to identify other senescence biomarkers, which limits its usefulness in the development of senolytic therapies. Additionally, colorimetric X-Gal staining is only useful for brightfield microscopy applications; it cannot be combined with fluorescence immunostaining or detected by flow cytometry. The fluorogenic substrate C₁₂FDG (5-dodecanoylamino fluorescein di- β -D-galactopyranoside) has also been used for β -Gal detection; however, its fluorescent product is prone to leakage from the cell, making it incompatible with immunostaining protocols.

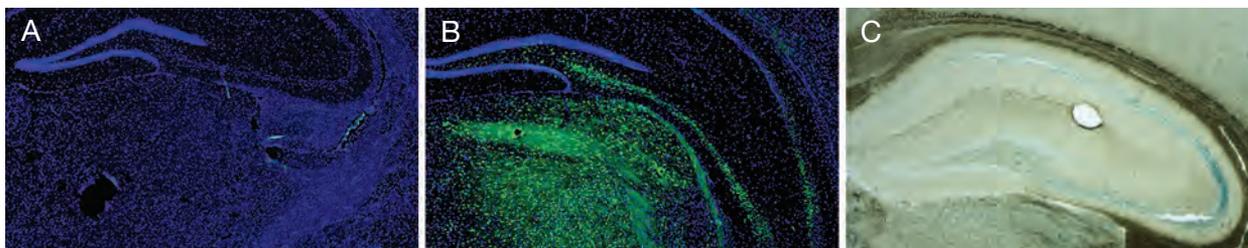


Figure 2. Cleavage of the CellEvent Senescence Green β -Gal substrate results in covalently bound green-fluorescent product. Frozen brain tissue from 6-month-old (A) wild-type and (B) Nrf2-knockout mice was cut into 10 μ m sections, fixed in 2% formaldehyde/0.8% glutaraldehyde in PBS, rinsed in PBS, and stained using the Invitrogen™ CellEvent™ Senescence Green Detection Kit (Cat. No. C10850) at pH 5 for 4 hr at 37°C without CO₂. After incubation, the tissue was washed with PBS and mounted with Invitrogen™ ProLong™ Gold Antifade Mountant with DAPI (Cat. No. P36935), then imaged on the Invitrogen™ EVOS™ M7000 Imaging System at 4x magnification using filters appropriate for DAPI and GFP. There is an increase in senescent cells in the hippocampus of the Nrf2 knockout, as shown by staining with the CellEvent Senescence Green Probe. (C) Tissue from a Nrf2-knockout mouse was also stained with X-Gal overnight and imaged using brightfield microscopy. Both CellEvent Senescence Green and X-Gal staining show a band of β -Gal-positive cells visible in the CA3 region. Data used with permission from Viviana Pérez, Oregon State University, Corvallis, Oregon.

CellEvent Senescence Green: A fluorogenic senescence-associated β -Gal probe

To address the limitations of current senescence detection methods, we have developed the Invitrogen™ CellEvent™ Senescence Green Probe. This fluorogenic β -Gal substrate is designed for the detection of senescent cells by fluorescence imaging or flow cytometry, based on the upregulation of SA β -Gal (Figure 1). The CellEvent Senescence Green Probe contains two galactoside moieties, as well as an additional moiety that reacts with several functional groups found in proteins. This nonfluorescent substrate is cleaved by intracellular β -Gal to produce a bright green-fluorescent product (Ex/Em = 490/514 nm) that is well retained in cells due to its covalent binding to intracellular proteins. In addition, the CellEvent Senescence Green Probe is easy to use: simply fix the cells, add the reagent, incubate, and detect fluorescence by imaging or flow cytometry.

CellEvent Senescence Green Probe for drug discovery

In pursuit of both therapeutic targets and senolytic drugs for the treatment of age-related neurodegenerative diseases, Viviana Pérez and her team at Oregon State University are studying how rapamycin, a compound found to increase longevity and improve health in several species, may exert its effect by inhibiting cellular senescence through proteins such as Nrf2 [5]. Nrf2 is a transcription factor that regulates cellular protection genes, and altered Nrf2 function is found in many neurodegenerative diseases, making Nrf2 a potential therapeutic target. The Pérez lab has recently used the CellEvent Senescence Green Probe to show that there is an increase in senescent cells in

the hippocampus of Nrf2-knockout mice, a mouse model previously shown to have premature senescence (Figure 2). The ability to stain tissues with the CellEvent Senescence Green Probe has allowed the Pérez lab to detect senescent cells in brain tissue more quickly and multiplex this fluorogenic SA β -Gal substrate with other fluorescent markers (data not shown).

Incorporate the CellEvent Senescence Green Probe in your senescence assays

The CellEvent Senescence Green Probe provides a simple, easy-to-use fluorogenic β -Gal substrate for identifying senescent cells, can be multiplexed with antibodies and other fluorescent cell health probes, and facilitates the identification of senescence pathway proteins that can be targeted with senolytic drugs. Senescence research has the potential to profoundly affect the quality of life as humans age. Learn more about the CellEvent Senescence Green Probe at thermofisher.com/senescence. ■

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| Product | Quantity | Cat. No. |
|--|-------------|----------|
| CellEvent™ Senescence Green Detection Kit | 25 μ L | C10850 |
| | 100 μ L | C10851 |
| CellEvent™ Senescence Green Flow Cytometry Assay Kit | 50 assays | C10840 |
| | 200 assays | C10841 |

Acquire and analyze microplate data more quickly and easily

Enhanced SkanIt Software 6.0.1 for microplate readers.

Like many cell biology labs, those focused on neuroscience research rely on microplate readers. From measuring neuronal viability to studying neuronal mechanisms that lead to disease or determining the concentration of neuronal proteins, microplate readers are foundational instruments that enable the quantification of signals generated in many different types of microplate assays.

While the actual instrument performance of microplate readers differs very little between instrument suppliers, the software programs that operate the readers can provide highly contrasting experiences. Thermo Scientific™ microplate readers are operated by Thermo Scientific™ SkanIt™ Software, which offers a remarkably smooth user experience. With the release of SkanIt Software 6.0.1 in April 2019, this experience is even more straightforward and accommodating. Neuroscientists can acquire their data with a microplate reader control-and-analysis interface that not only is simple to use but also aids with error prevention, helps boost productivity, and can easily be used in conjunction with tools already essential in research labs, such as Microsoft™ Excel™ data analysis software. Here we discuss a few examples of the distinctive time-saving, hassle-free features offered by SkanIt software. These helpful features span the entire plate reading workflow, from protocol setup through execution to data analysis.

Preconfigured protocols that are ready to use right off the shelf

Instead of requiring you to set up your protocols from scratch, SkanIt software provides access to the prebuilt SkanIt Cloud Library of assay protocols. This collection of validated sessions contains representative protocols with measurement steps (and real data), as well as calculations and other analysis steps associated with typical bioassays, using the most common optical detection technologies: absorbance, fluorescence, time-resolved fluorescence, luminescence, and AlphaScreen (Amplified Luminescent Proximity Homogeneous Assay Screen) (Figure 1). Users can access this SkanIt Cloud Library to perform immediate measurements, or they can use the sessions as templates to create customized assay protocols. The SkanIt Cloud Library is also an excellent data source for training (including self-training) of users new to SkanIt software. The library currently contains more

A

| Assay type | Detection technology (examples) |
|------------------------------|---|
| Quantification of dsDNA | Photometry (UV absorbance) |
| | Fluorescence (Invitrogen Quant-iT assays) |
| Quantification of RNA | Photometry (UV absorbance) |
| | Fluorescence (Invitrogen Quant-iT assays) |
| Quantification of proteins | Photometry (UV absorbance) |
| Colorimetric protein assays | Photometry (Bradford and Lowry assays) |
| Cell health | Photometry (Invitrogen Vybrant MTT assay) |
| | Fluorescence (Invitrogen PrestoBlue and alamarBlue assays) |
| | Luminescence (Promega CellTiter-Glo and Caspase-Glo 3/7 assays) |
| Reporter assays | Luminescence (Promega Dual-Luciferase reporter assays) |
| Protein-protein interactions | Luminescence (Promega NanoBRET PPI assays) |
| Ca ²⁺ flux assays | Fluorescence (fluo-4 and fura-2 assays) |

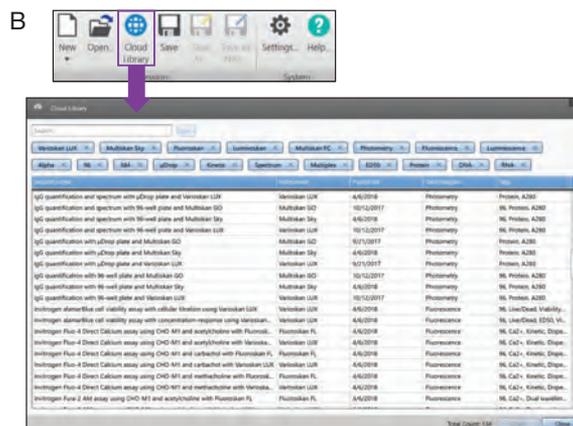


Figure 1. Execute microplate measurements more easily with Thermo Scientific microplate readers. The Thermo Scientific™ SkanIt™ Cloud Library offers a ready-to-use collection of relevant assays that can be either applied as-is or customized with quick editing. **(A)** Representative examples of assays included in the SkanIt Cloud Library. **(B)** A screenshot of the SkanIt Cloud Library, shown after selecting the Cloud Library icon from the SkanIt software Home menu.

than 130 instrument-specific assays and can be quickly searched with customized tags. The SkanIt Cloud Library will continue to expand as new applications emerge.

Hassle-free measurements with Automatic Dynamic Range

Setting up fluorescence or luminescence assays using a microplate reader that requires manual adjustment of its gain setting is a

cumbersome process. For example, if the effect of a certain neurotoxic compound is being studied over a broad concentration range, it can be expected that the signal will vary significantly across the measured wells. With instruments that require manual gain adjustment, finding the gain setting that will produce the optimal instrument response is time-consuming and frustrating. High-concentration samples can saturate instrument optics and require a lower gain setting, whereas low-concentration samples may not be easily detectable and require a higher gain setting.

In contrast, SkanIt software includes an Automatic Dynamic Range (ADR) feature for performing fluorescence and luminescence measurements with the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader. By selecting ADR, the instrument automatically adjusts the optimal gain setting based on the signal intensity in each well. As a result, using the ADR with the Varioskan LUX reader significantly broadens the quantifiable range of assays, compared to instruments requiring manual gain setting (Figure 2). Because ADR acts quickly in real time, the gain is set to its optimal value in each well, even when users are performing kinetic or spectral measurements. This distinctive feature, available exclusively for the Varioskan LUX reader, greatly simplifies the optimization of assays and makes the data comparable from run to run.

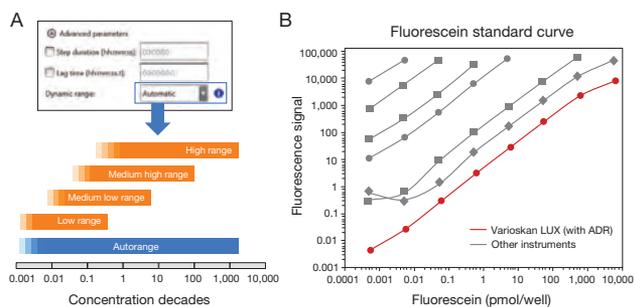


Figure 2. Don't bother with manual gain settings. Selecting Automatic Dynamic Range (ADR) in Thermo Scientific™ SkanIt™ Software helps save time and reduce effort when measuring fluorescence and luminescence assays with the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader. **(A)** A screenshot shows the selection of ADR in SkanIt software. **(B)** Instead of having to perform several measurements with different gains (as required on instruments from other suppliers), optimal results can be obtained after a single measurement using the Varioskan LUX microplate reader with ADR, as shown in this example of a fluorescein standard curve obtained on several instruments using different gain settings.

| Product | Quantity | Cat. No. |
|---|----------|----------|
| SkanIt™ Software for Microplate Readers, Research Edition | 1 each | 5187139 |
| SkanIt™ Software Upgrade Package, Research Edition (for Fluoroskan™, Fluoroskan™ FL, Fluoroskan™ Ascent, Fluoroskan™ Ascent FL, Luminoskan™, and Luminoskan™ Ascent microplate reader models) | 1 each | N17646 |
| Varioskan™ LUX Multimode Microplate Reader (absorbance, fluorescence intensity, and luminescence measurements; top- and bottom-reading options) | 1 each | VLBLATDO |

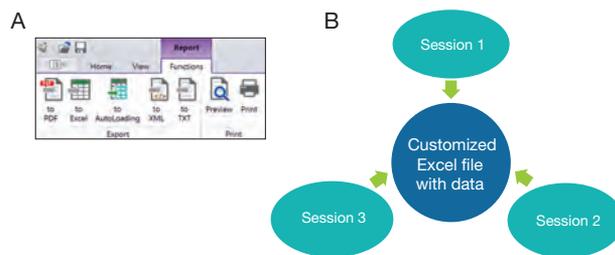


Figure 3. Conveniently autoload data into Excel software. With the Excel AutoLoading feature, the measured or calculated data from Thermo Scientific™ SkanIt™ Software can be mapped to a customized Microsoft™ Excel™ template. **(A)** This screenshot shows the AutoLoading option in SkanIt software. **(B)** The AutoLoading feature allows the results from multiple experiments to be combined into a single Excel file, facilitating the use of customized and shared Excel templates.

Conveniently send data directly to customized Excel files using the AutoLoading tool

Post-measurement processing of data in Excel software is the most common practice when analyzing the results of a microplate assay. While all SkanIt software versions have single-click export to Excel software, SkanIt Software 6.0.1 includes an Excel AutoLoading tool for additional convenience. This new feature supports automatic loading of data from SkanIt software into a specific predesigned target Excel file (Figure 3). This tool is especially convenient for users who:

- Are accustomed to using specific Excel files to make calculations
 - Use shared data templates within their research group
 - Wish to combine data from multiple experiments in Excel software
- Using Excel AutoLoading, SkanIt calculation steps can also be easily exported to custom Excel templates. This convenient tool is one more way SkanIt software enables easy reporting and analysis.

Take advantage of the newest SkanIt software features

SkanIt software gives you full control over the instrument settings for all Thermo Scientific microplate readers, supporting the optimal use of instrument features and offering visual workflow setup and effortless data reduction and exporting. Find additional features and capabilities of the recently launched SkanIt Software 6.0.1 at thermofisher.com/skanit. ■

Modeling antitumor responses with spheroid-immune cell cocultures

Cocultures of human colorectal tumor spheroids with immune cells reveal the therapeutic potential of MICA/B and NKG2A targeting for cancer treatment

Courau T, Bonnereau J, Chicoteau J, Bottois H, Remark R, Assante Miranda L, Toubert A, Blerly M, Aparicio T, Allez M, Le Bourhis L (2019) *J Immunother Cancer* 7:74.

Researchers have yet to find effective immunotherapies to treat patients afflicted with colorectal cancer, in part due to the lack of accessible models for identifying key effectors and inhibitors that might serve as appropriate antibody targets. In their recent publication, Le Bourhis and his team at INSERM in Paris describe the preparation of colorectal cancer tumor spheroids and their coculture with allogenic immune cells, including T cells and NK cells, that rapidly infiltrate the cell line-derived spheroids. They demonstrate the use of these cocultures to study anti-tumor immune responses such as tumor cell apoptosis and spheroid destruction *in vitro*, and further confirm their observations *ex vivo*.

By producing individual, uniform spheroids in matrix- and growth factor-free medium, these researchers were able to precisely and reproducibly manipulate coculture settings, as well as monitor immune cell infiltration, measure spheroid volume, and assess tumor cell apoptosis by flow cytometry and fluorescence imaging (Figure 1). Their characterization of the infiltrating (IN) and non-infiltrating (OUT) immune cells showed that memory T cells and NK cells induce tumor cell destruction via interferon and NKG2D-MICA/B pathways.

Based on these observations, this team evaluated antibodies specific to NKG2D, an activator of cytotoxic responses, and its ligands MICA and MICB. When they treated cocultures with anti-MICA/B antibody, spheroid destruction by NK cell infiltration and activation was enhanced. They also observed the upregulation of HLA-E (a ligand of the inhibitory NKG2A receptor) on tumor cells and an increase in NKG2A expression on infiltrating NK cells, leading to an assessment of the effects of anti-NKG2A antibody. Although treatment of cocultures with anti-NKG2A antibody alone did not show a significant response, the combined treatment with anti-MICA/B antibodies provided a coordinated, synergistic antitumor effect, implicating both the NKG2D-MICA/B and NKG2A-HLA-E pathways in immune-mediated tumor disruption. These observations were confirmed using spheroids derived directly from primary tumors of colorectal cancer patients in conjunction with autologous tumor-infiltrating lymphocytes. This work illustrates the utility of tumor spheroids as a relevant and representative model for defining the interactions between tumor cells and lymphocytes in the effort to identify critically needed cancer immunotherapies. ■

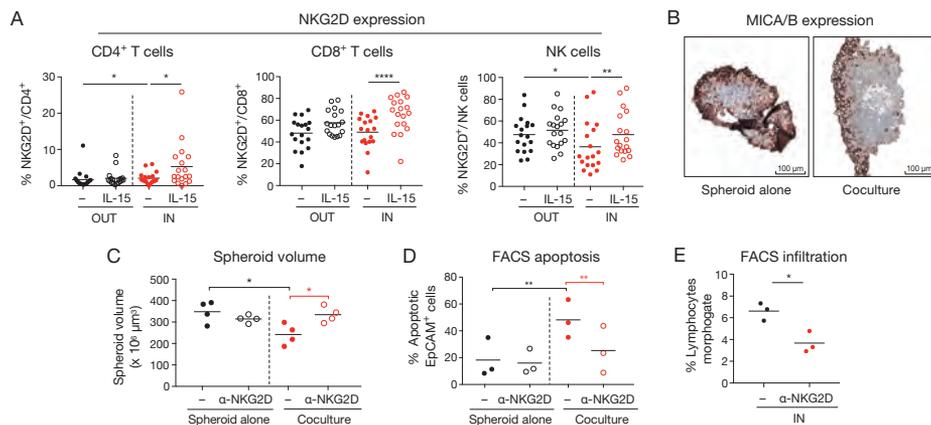


Figure 1. NKG2D-MICA/B pathway is engaged in cocultures. (A) NKG2D expression by CD4 T cells, CD8 T cells, and NK cells in the IN (infiltrating cells isolated from sedimented, rinsed, trypsinized spheroids) and OUT (non-infiltrating cells) compartments, in the presence or not of IL-15, at 24 hr as measured using the Invitrogen™ Attune™ NxT Flow Cytometer; n = 18 independent experiments. (B) MICA/B expression by tumor cells in the spheroids cocultured or not with CD19-CD14⁺ PBMCs, as measured by immunohistochemistry at 24 hr; representative images of 1 experiment, acquired using the Invitrogen™ EVOS™ FL Cell Imaging System. Analyses of (C) spheroid volume, (D) tumor cell apoptosis, using Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent (Cat. No. C10423), and (E) spheroid infiltration 48 hr after coculturing HT29 spheroids with CD19-CD14⁺ PBMCs in the presence or not of anti-NKG2D blocking antibodies; n = 3–4 independent experiments. Statistical significance of A and C–E were analyzed using the Wilcoxon matched-pairs signed rank test and the paired *t* test, respectively (* *p* < 0.05, ** *p* < 0.005, *** *p* < 0.001, **** *p* < 0.0001). Reproduced with permission from Courau T et al. (2019) *J Immunother Cancer* 7:74, and under the Creative Commons Attribution 4.0 International License (creativecommons.org/licenses/by/4.0/).

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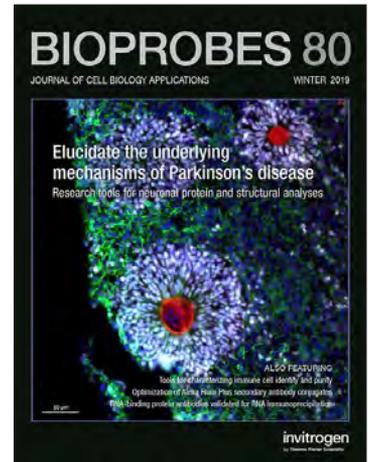
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Cover image

Immunofluorescence analysis of ZO-1 (red) and SOX2 (gray) in human iPSC-derived forebrain organoids. At day 40, organoids were fixed with 4% formaldehyde for 1 hr at room temperature and incubated in a 30% sucrose solution overnight at 4°C. The organoids were then embedded, cryosectioned at 5 µm, permeabilized with 0.2% Triton™ X-100 for 20 min, and blocked with 10% donkey serum in PBS for 30 min at room temperature. Organoid slices were incubated with a 1:500 dilution of Invitrogen™ ZO-1 mouse monoclonal antibody (clone ZO1-1A12, Cat. No. 33-9100) and Invitrogen™ SOX2 rabbit polyclonal antibody (Cat. No. PA1-094X), and a 1:1,000 dilution of a MAP2 chicken polyclonal antibody in blocking buffer overnight at 4°C. Samples were then stained with a 1:1,000 dilution of Invitrogen™ Alexa Fluor™ 568 donkey anti-mouse IgG (red, Cat. No. A10037), Invitrogen™ Alexa Fluor™ 647 donkey anti-rabbit IgG (gray, Cat. No. A31573), and Alexa Fluor™ 488 donkey anti-chicken IgG (green) secondary antibodies, as well as DAPI (blue), in blocking solution at room temperature for 1 hr. Images were taken on a Nikon™ Inverted Eclipse™ Ti-E Microscope at 20x magnification. Scale bar: 50 µm. Used with permission from Zhexing Wen, Emory University School of Medicine, Atlanta, Georgia.



Previous issues



BIOPROBES 79

This issue features adoptive cell therapies in immuno-oncology research and the use of cytotoxicity assays with 3D tumor models. Also discussed are best practices for multiparameter flow cytometry, antibodies and immunoassays for signaling pathway research, and functional assays for senescence, drug-induced calcium flux, microtubule dynamics, and biofilm cell viability.



BIOPROBES 78

In this issue, we describe media, cultureware, and reagents for 2D and 3D neuronal cell culture and fluorescence imaging, as well as time-saving immunoassays such as Invitrogen™ Instant ELISA™ kits, ProQuantum™ kits, and ProcartaPlex™ multiplex panels. We also highlight the Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer and the Invitrogen™ Attune™ NxT Autosampler for high-throughput assays.



BIOPROBES 77

This issue focuses on advances in immuno-oncology such as immune checkpoint inhibitors and T cell immunotherapy, and the use of stem cell differentiation models to verify antibody specificity. Also described are tools for flow cytometry and high-content imaging and analysis, as well as Invitrogen™ iWestern™ workflow instrumentation and the updated Invitrogen™ Qubit™ 4 Fluorometer and Qubit™ RNA IQ Assay Kit.

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