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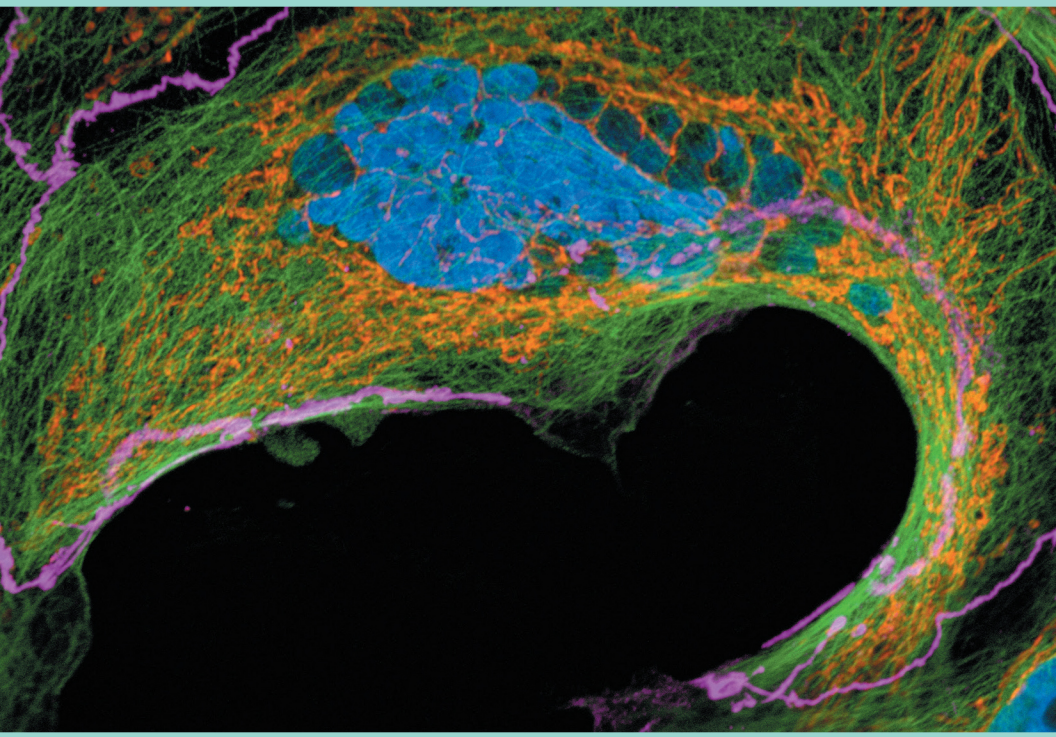

Antibody Validation

Essential Knowledge Briefings

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COVER IMAGE CAPTION: Human epithelial colorectal adenocarcinoma cells (Caco-2) labeled with a beta tubulin primary antibody directly conjugated to Invitrogen™ Alexa Fluor™ 488 (green) dye, ATP synthase beta primary antibody directly conjugated to Invitrogen™ Alexa Fluor™ 555 (orange) dye, ZO-1 primary antibody directly conjugated to Invitrogen™ Alexa Fluor™ 647 (magenta) dye, and Invitrogen™ NucBlue™ Fixed Cell Stain for nuclei (blue). Image stack was acquired on the Zeiss 710 laser scanning confocal microscope and displayed as a maximum-intensity projection.

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When stating Thermo Fisher products or methods: 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.

Prepared for Wiley and Thermo Fisher Scientific, Inc. by EKB Editor, Mike May, Ph.D.

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INTRODUCTION

Scientific research, across a range of applications, suffers from an antibody crisis. "Antibodies are among the most frequently used tools in basic science research and in clinical assays, but there are no universally accepted guidelines or standardized methods for determining the validity of these reagents," wrote David Rimm, professor of pathology and medicine at Yale University, and his colleagues (https://www.biotechniques.com/biotechniques/BiotechniquesJournal/2010/March/Antibody-validation/biotechniques-200748.html). "Furthermore, for commercially available antibodies, it is clear that what is on the label does not necessarily correspond to what is in the tube." Consequently, this reagent crisis has emerged from a lack of consistent and effective antibody validation, creating grave financial and scientific consequences.

Professor Rimm understands the depth, danger, and expense of the problem from personal experience. In 2009, he found that a new supply of antibodies—from the same supplier that he'd been using previously—didn't function in the same way as those that he had purchased before, and furthermore, he'd included the previous studies as evidence that had earned him $2 million of funding for an antibody-based method to analyze tumor biopsies. Instead of moving toward what he expected to be an exciting result, the conflicting results drove him to terminate the project.

Rimm's experience doesn't stand alone. In fact, many scientists suffer from similar circumstances. For example, Ioannis Prassas and Eleftherios Diamandis—both experts in cancer research at Mount Sinai Hospital in Toronto—spent about two years and $500,000 working with a commercial enzyme-linked immunosorbent assay (ELISA) kit marketed to quantify CUB and Zona Pellucida-Like Domains 1 (CUZD1) protein. However, the experiment didn't work because the kit, instead, targeted an ovarian cancer antigen, CA125 (https://www.degruyter.com/view/j/cclm.2014.52.issue-6/cclm-2013-1078/cclm-2013-1078.xml).

As Prassas and Diamandis wrote: "While we do not know how this could have happened during manufacturing, the consequences of such errors can be quite severe." Regardless of the details that created this problem, poor antibody validation was certainly an influencing factor.

Although these are specific examples, the antibody crisis is widespread. In 2016, Mathias Uhlén, professor of microbiology at the Royal Institute of Technology (Stockholm, Sweden), and his colleagues formed an "*ad hoc* International Working Group for Antibody Validation in order to formulate the best approaches for validating antibodies used in common research applications and to provide guidelines that ensure antibody reproducibility." (http://www.nature.com/nmeth/journal/v13/n10/full/nmeth.3995.html) They reported that the "extensive discussion of antibody validation in the literature indicates a collective need for standards to validate antibody specificity and reproducibility, as well as a need for adequate reporting practices." From this work, this group proposed five pillars for validating antibodies: genetic strategies, orthogonal strategies, independent antibody strategies, expression of tagged proteins, and immunocapture followed by mass spectrometry.

In December 2016, the problem of antibody performance spurred The Global Biological Standards Institute (GBSI) and The Antibody Society to gather antibody producers, researchers, and publishers at the Asilomar Conference Grounds. The organizers intended to "share perspectives and contribute to tangible solutions for validating antibodies." Therefore, the "Asilomar Antibody Workshop Report" concluded: "A single set of community-accepted validation guidelines and standards that is sufficiently comprehensive and accessible to the entire biological research community has not emerged to date." The groups supporting this conference—including the US National Institutes of Health (NIH), industrial leaders such as Thermo Fisher Scientific, and others—reflect the overall interest in antibody validation.

As these examples show, many scientists, government agencies, and suppliers understand the breadth of the antibody crisis. And these experts realize that antibodies failing to perform as indicated cost researchers time and money. Moreover, the amount of money lost is staggering. In 2015, Andrew Bradbury—a group leader and scientist in the bioscience division at the Los Alamos National Laboratory, New Mexico—and Andreas Plückthun—professor and head of the biochemistry department of the University of Zurich, Switzerland—plus 110 co-signatories, reported that improperly or poorly performing antibodies could waste up to $350 million a year in the United States (https://www.nature.com/news/reproducibility-standardize-antibodies-used-in-research-1.16827). Beyond the squandered time in the lab and the wasted funding dollars, opportunities could be missed and incorrect research paths followed. In short, antibody validation constitutes a crucial element of today's life science research and health care.

Although this metric of lost research dollars—$350 million per year in just one country—seems bad enough, it could get much worse. According to Transparency Market Research, the global market for antibodies was $86.7 billion in 2015, and will climb to $245.8 billion by 2024. With those numbers, even a fraction of the antibodies failing to perform as expected wastes large amounts of research funding.

To address this issue, suppliers must develop and consistently perform antibody-validation procedures. For example, Thermo Fisher Scientific uses a two-part approach that validates each antibody's target specificity and performance in functional applications (see "A two-part process" section below). To promote transparency, the details of these processes will be made publicly available. Overall, properly validated antibodies impact a broad range of stakeholders, including antibody producers, basic- and applied-science researchers, funding agencies, health care workers, pharmaceutical companies, publishers, and patients. Only when all suppliers perform the necessary quality control on antibodies can scientists move forward with reliable and reproducible research.

VALIDATION DETAILS

The natural role of antibodies binding to specific target molecules creates a powerful research tool with the ability to locate and quantify particular molecules. A primary antibody binds to a specific protein. A secondary antibody—which is usually conjugated to a label, such as Alexa Fluor dye—binds to a primary antibody. To be an effective tool, an antibody's performance must be confirmed.

Research confirms the extent of the problem that arises from vendors failing to validate antibodies. In a 2008 report, Uhlén and his colleagues collected about 5,500 antibodies against human proteins from 51 suppliers (http://www.mcponline.org/content/7/10/2019.abstract). The testing performed by Uhlén's team found that only about half of these antibodies stained the intended target in western blot (WB) or immunohistochemistry (IHC). The researchers noted in their report: "The success rates stratified by the different providers showed large differences, ranging from 0 to 100% of the antibodies." Moreover, monoclonal and polyclonal antibodies from the same suppliers varied dramatically—one class of antibodies often being twice as likely to perform as expected compared with the other class. The authors did note: "It is important to point out that many of these antibodies have not been approved by the antibody providers for immunohistochemistry; this might explain the low success rate in our hands." For antibody-based research to produce reliable results, scientists depend on all reagents working as expected; yet even the same commercial antibody from some individual suppliers can vary from one batch to the next.

A validated antibody meets two general criteria. First, it must bind to a specific target. Second, a validated antibody works in a given application. Part of the challenge arises from the wide range of applications of antibodies. Antibodies are used to identify proteins with ELISAs, IHC, immunoprecipitation (IP), quantitative

immunofluorescence (QIF), and WB (Figure 1). For example, IHC is used to test for human epidermal growth factor receptor 2 (HER2) in breast cancer samples. In basic research, scientists use antibodies to determine the presence of a specific target and to select and purify it for further analysis.

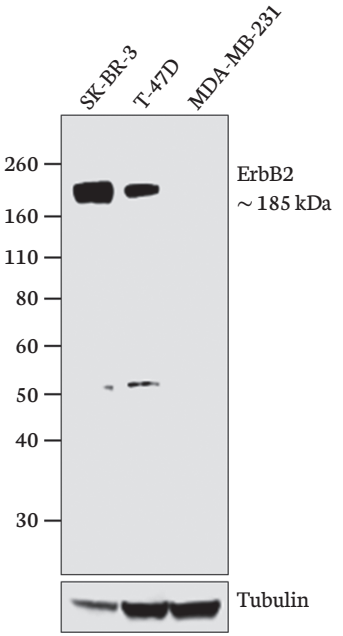

Figure 1. Western blot analysis was performed on membrane enriched extracts (30 µg lysate) of SK-BR-3 (Lane 1), T-47D (Lane 2) and MDA-MB-231 (Lane 3). The blot was probed with Invitrogen™ ErbB2 (HER-2) Polyclonal Antibody (Product # PA5-16305, 1 µg/ml) and detected by chemiluminescence using Invitrogen™ Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.25 µg/ml, 1:4,000 dilution). A 185 kDa band corresponding to ErbB2 was strongly expressed in SK-BR-3, moderately expressed in T-47D, and not expressed in MDA-MB-231, which is an ErbB2-negative cell line. which is an ErbB2 negative cell line. Known quantities of protein samples were electrophoresed using Invitrogen™ NuPAGE™ 4–12% Bis-Tris Gel (Product # NP0321BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Sharp Pre-Stained Protein Standard (Product # LC5800). Resoalved proteins were then transferred onto a nitrocellulose membrane using the wet transfer system. The membrane was probed with the relevant primary and secondary antibodies following blocking with 5% skimmed milk. Chemiluminescent detection was performed using Invitrogen™ ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). (Image courtesy of Thermo Fisher Scientific.)

Moreover, many details of an application—from the organism used to make the antibody, to the organism being tested, and other variables in a protocol—can impact an antibody's performance. Even the type of tissue matters. In some cases, an antibody created from, say, rat muscle will not work in all rat tissues, let alone tissues from different species. The treatment of the tissue—such as formalin-fixed, paraffin-embedded (FFPE) preparation—can also impact the function of an antibody. In addition, as Uhlén and his colleagues noted: "Antibody performance is highly dependent on target protein concentration."

Some applications pose even more challenges for antibodies. With IHC, for example, the antigenicity of a tissue and identifying a target in the tissue can depend on many factors, including how the tissue is prepared and analyzed, respectively. Even the time of fixation of the tissue, inadequate fixing, and the fixative used can impact the results. In IHC, the challenges run even deeper to the specific antigen and its dilution.

Moreover, the use of antibodies impacts downstream applications. In developing a biomarker for a clinical test, for example, scientists use IHC to validate the biomarker. Therefore, a poorly performing antibody can drastically slow down the process of moving a biomarker-based test from a research lab to a clinically approved method. Even worse, it can take years and considerable work to determine that the antibody is the source of the problem. In addition, this is just one of many steps involved in moving from preclinical to clinical tests.

The techniques for validating antibody-based methods also evolve over time. As an example, biomedical engineer Evgin Goceri of Akdeniz University in Turkey and her colleagues at The Ohio State University used imaging to validate an antibody to polypyrimidine tract-binding protein 1 (PTBP1) for use in diagnostic neuropathology (http://onlinelibrary.wiley.com/doi/10.1002/cnm.2862/full) (Figure 2). To do this, the scientists prepared images of neuropathology samples stained with anti-PTBP1 antibody, removed noise with a computational algorithm, normalized the images with a sigma-adaptive Gaussian filter, and detected the cell nuclei. "Experiments on 29 data sets from 3 cases of brain tumor and reactive gliosis show statistically significant differences between the number of positively stained nuclei in images stained with and without anti-PTBP1 antibody," they wrote. "The experimental analysis of specimens from 3 different brain tumor groups and 1 reactive gliosis group indicates the feasibility of using anti-PTBP1 antibody in diagnostic neuropathology, and computerized image analysis provides a systematic and quantitative approach to explore feasibility."

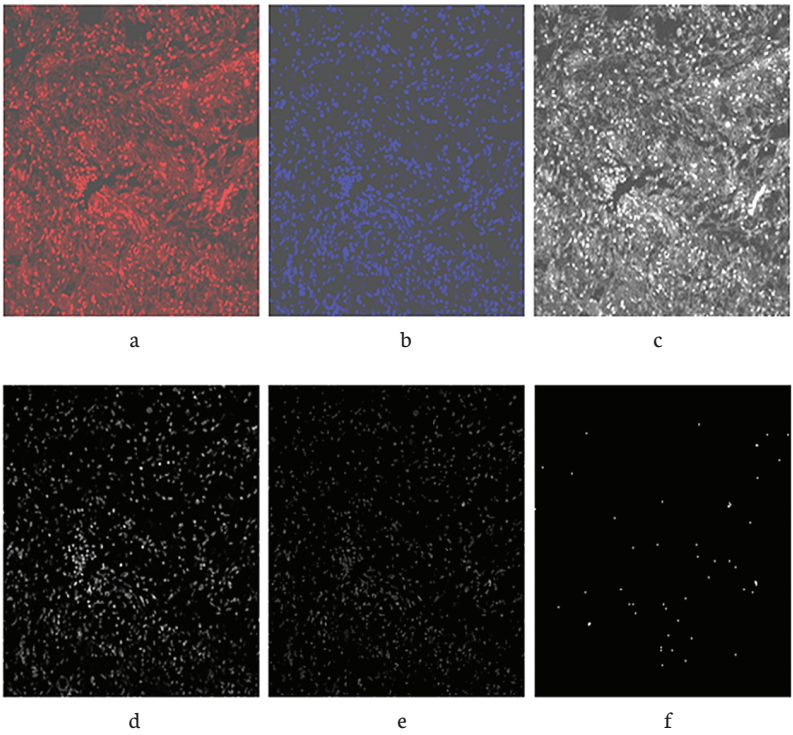

Figure 2. An image stained with anti-PTBP1 antibody (primary) from pilocytic astrocytoma (PA) (a) Image stained with 4',6-diamidino-2-phenylindole (DAPI) from PA. (b) Grayscale forms of the image stained with anti-PTBP1 (polypyrimidine tract-binding protein 1) antibody. (c) DAPI. (d) Generated *DAPI_PTBP1* image. (e) Nuclei positively stained with anti-PTBP1 antibody (to better visualize images in A–C, brightness and contrast have been increased by 30%.) (f) [From: Goceri, E. et al. (2017). Quantitative validation of anti-PTBP1 antibody for diagnostic neuropathology use: Image analysis approach. *International Journal for Numerical Methods in Biomedical Engineering*, (epub 10 February 2017).]

In the face of this challenging and evolving area of science, concerned stakeholders have implemented proactive processes for validating antibodies. Some antibody producers already run sophisticated validation operations. In addition, an increasing number of journals require authors to follow strict rules related to antibodies used in research, including reporting all specific existing information. To resolve this problem, however, all vendors and journals must take action.

LAB TIPS

Even in the midst of the antibody crisis, scientists must still use these tools. Because of this, it pays to follow a few best-practice tips in selecting an antibody. As Andrew Chalmers, senior lecturer in cancer research at the UK-based University of Bath, wrote: "The task of selecting an antibody is far from straightforward and . . . several existing antibody search engines have been developed to help this process." (https://bmccellbiol.biomedcentral.com/articles/10.1186/1471-2121-15-6) Beyond turning to a search engine, scientists can rely on a few selection guidelines.

First, avoid the temptation to use an antibody that is supposed to perform many functions. Instead, pick an antibody that was designed and developed for a specific application and research setup.

Second, consider how long an antibody will be used. For applications that will use the same antibody across multiple years and purchases, a monoclonal antibody could be somewhat more consistent than a polyclonal antibody.

Third, and as already noted but worth repeating, make sure that the antibody has been tested against the intended target and application. In addition, find a supplier who provides data to back up those validation procedures, plus information about the cell lines and experimental controls used.

Fourth, check to see if scientists have used the antibody in publications that addressed similar research objectives. Although this doesn't ensure that an antibody will perform as desired in a particular application, it provides one more piece of information.

In fact, the most important thing is to collect as much information about an antibody as possible before using it. Regardless of the supplier, insist on receiving data about using the antibody in a specific application; but note that suppliers are often reluctant to provide

that evidence. Consequently, researchers must also test an antibody's performance in the study in which it will be used. This puts the burden on both suppliers and scientists.

The method of making an antibody—developed against a natural or synthetic protein—can also impact its specificity. The synthetic proteins offer some benefits as an antibody source, because the antibody producer knows the amino-acid sequence in which the antibody binds. Nonetheless, a synthetic protein might not fold into the same three-dimensional structure that a natural protein does. Also, the synthetic protein lacks the posttranslational modifications that exist in natural proteins. So, an antibody raised against a synthetic version of a protein might not work as well in applications, such as IP and IHC, that keep a protein in its three-dimensional form. When the target protein is denatured, such as in WB, the antibody made from a synthetic protein might work as intended. Conversely, making antibodies from purified proteins might work very well in applications that keep the target protein in its natural shape, but not as well when it's denatured.

Scientists should carefully track the antibodies being used. When purchasing an antibody, researchers should record the lot numbers and data on the product from the supplier, which should include information on a specific antibody's intended applications and conditions. To compare studies from the same technique over time, an antibody must perform consistently. Comparisons of data from different methods also rely on reproducible antibody performance.

A TWO-PART PROCESS

At Thermo Fisher Scientific, Invitrogen™ antibodies are going through a rigorous, two-part verification process in order to give scientists confidence in the quality of their antibodies.

Part one of the process is target-specificity verification, which confirms that an antibody binds to the intended target. For each antibody, one of nine testing methods is used to ensure specificity. IP-mass spectrometry (IP-MS) identifies antibody targets with IP followed by MS. Genetic-modification techniques include knockout methods, such as CRISPR-Cas9 cell models (Figure 3), or knockdown techniques, such as RNA interference (RNAi) (Figure 4), which knock down a target gene. Independent antibody verification (IAV) uses two antibodies developed for different parts of the same target. Biological verification can be tested in five ways: downstream events can be analyzed following a cell treatment; naturally occurring variable expression of a target molecule can be used to validate an

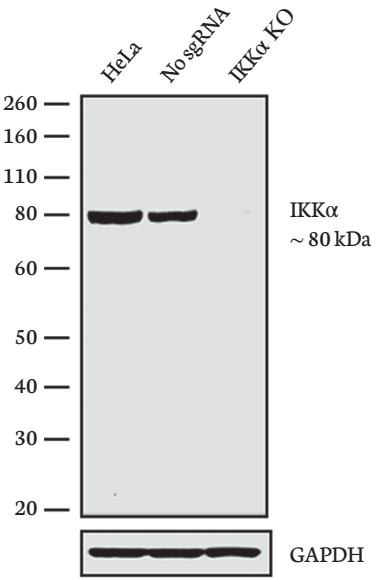

Figure 3. Western blot analysis of IKK alpha was performed by loading 30 micrograms of HeLa (lane 1), ME180 (lane 2), ME180-IKK alpha knockout (lane 3) whole cell lysate using a NuPAGE 4–12% Bis-Tris Gel (Product # NP0321BOX), the XCell SureLock Electrophoresis System (EI0002), the Sharp Pre-Stained Protein Standard (LC5800), and the Invitrogen™ iBlot™ Gel Transfer Device (IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5% skim milk for 1 hour at room temperature. IKK alpha was detected at approximately 80 kDa using Invitrogen™ IKK alpha Rabbit Polyclonal Antibody (PA5-17803) at 1:1,000 in 5% skim milk at 4°C overnight on a rocking platform. Goat anti-Rabbit IgG (H+L) Superclonal Secondary Antibody, HRP conjugate (Product # A27036, 0.25 µg/ml, 1:4,000 dilution) was used and chemiluminescent detection was performed using the ECL Chemiluminescent Substrate Reagent Kit (WP20005). Loss of signal upon CRISPR-mediated knockout (KO) confirms that the antibody is specific to IKK alpha. (Image courtesy of Thermo Fisher Scientific.)

antibody's specificity; neutralization shows that an antibody blocks a protein by binding to it; peptide arrays test an antibody's reactivity against proteins with specific modifications; and orthogonal methods correlate target specificity from antibody-dependent and independent techniques.

The second part of the antibody validation process is functional validation, which confirms that an antibody works in specific applications. This includes chromatin immunoprecipitation (ChIP), flow cytometry, immunofluorescence (IF) imaging, IHC, WB, and others. If a scientist's specific application is not tested, the researcher must determine whether the antibody actually works as needed.

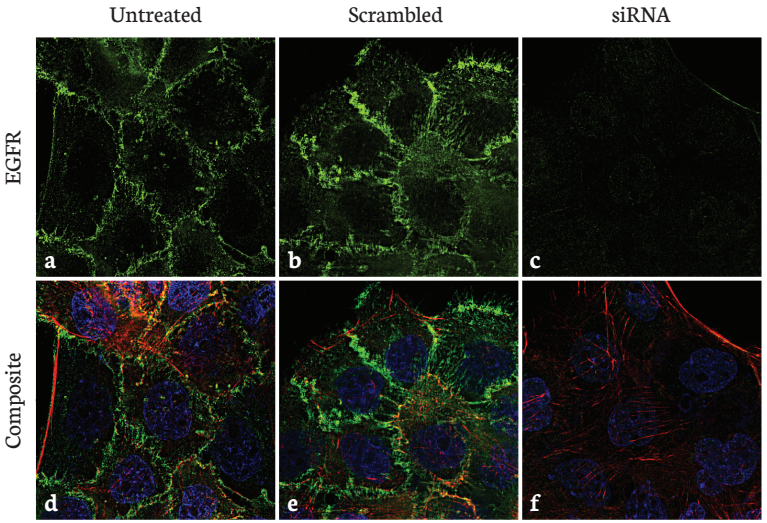

Figure 4. Knockdown of EGFR was achieved by transfecting A431 cells with EGFR-specific siRNA (Invitrogen™ Silencer ™ *Select* Product # s563, s564 and s565). Immunofluorescence analysis was performed on A431 cells (untransfected, panels a,d), transfected with nonspecific scrambled siRNA (panels b,e) and transfected with EGFR-specific siRNA (panels c,f). Cells were fixed, permeabilized, and labelled with Invitrogen™ Polyclonal Antibody (Product # PA1-1110, 5 µg/mL), followed by Invitrogen™ Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor 488 conjugate (Product # A27034, 1:2,000). Nuclei (blue) were stained using Invitrogen™ SlowFade™ Gold Antifade Mountant with DAPI (Product # S36938), and Invitrogen™ Rhodamine Phalloidin (Product # R415, 1:300) was used for cytoskeletal F-actin (red) staining. Loss of signal was observed upon siRNA-mediated knockdown (panels c,f) confirming specificity of the antibody to EGFR (green). The images were captured at 60x magnification. (Image courtesy of Thermo Fisher Scientific.)

An antibody often gets developed for one application. Thus performance in other applications must be confirmed to be effective.

This two-part process validates that an antibody specifically and selectively binds to its expected target and performs reproducibly in its intended application. Despite running its own internal antibody-validation process, Thermo Fisher Scientific also supports the International Working Group for Antibody Validation (IWGAV), which was created by an international team of scientists, with an intent to improve antibody performance. Some suppliers do not provide a transparent account of how they obtained the validation of their results in the first place. This is a major reason why scientists can't reproduce the findings of their suppliers.

VALIDATION APPROACH: CELL CYCLE STAGES

A crucial step in the eukaryotic cell cycle is the replication of chromosomes during the S phase. Studying this process provides one example of how Thermo Fisher Scientific validates antibodies.

Chromosome replication ensures that a complete set of genetic material is available for each daughter cell to inherit. Right after replication, sister chromatids are held together at their centromeres by a multimeric protein complex—the cohesion—until the metaphase-anaphase transition, when they segregate to the opposite poles of the dividing cell. Chromatid cohesion and segregation are tightly controlled in order to maintain genomic stability. The importance of this control is underscored by the numerous deadly effects of defective chromosome segregation resulting in aneuploidy or polyploidy, cancer, and drug resistance.

Cohesin was initially identified in ***Saccharomyces cerevisie*** and ***Xenopus***, and it is widely conserved among eukaryotes. In mammals, this complex consists of four proteins, one of which is STAG2. Centromeric cohesins form a ring-like structure around the centromere as early as the S phase. When vertebrate cells enter prophase, the cohesins start disengaging from the chromosomes in a mechanism that involves phosphorylation. Disassociation is complete in anaphase, when sister chromatids segregate.

In addition to its role in the cell cycle, the cohesin complex has also been implicated in gene repair and the control of transcription in mitotic and post-mitotic cells. Reliable antibodies against cohesin complex proteins that are functional in a wide range of applications are invaluable in understanding their functions.

The recombinant ABfinity STAG2 rabbit recombinant monoclonal antibody is functional in WB, immunofluorescence, and flow cytometry. In addition, advanced validation confirmed

its specificity with short interfering RNA (siRNA) treatment. Untransfected cells or cells transfected with scrambled siRNA showed abundant signal with the STAG2 antibody in WB and IF. By contrast, this signal was completely abolished in WB and IF when cells were transfected with STAG2-specific siRNA, confirming that the signal observed in both WB (Figure 5) and IF (Figure 6) is specific to STAG2.

The power of a superior antibody is the ability to use it in biologically appropriate experiments—fit for purpose—as a marker for endogenous cellular events. Consistent with the crucial role of STAG2 in the cohesin complex, its localization is altered during the course of the cell cycle (Figure 7). In interphase cells, STAG2 is distributed in

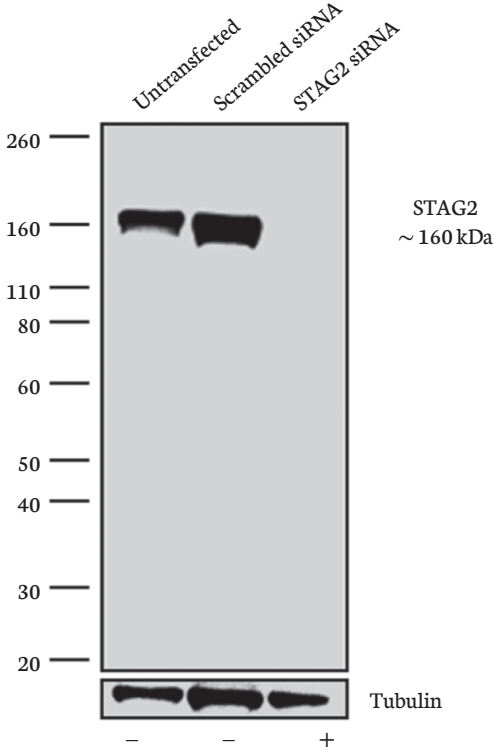

Figure 5. Detection of STAG2 in HeLa cells upon siRNA transfection using Invitrogen™ ABfinity™ STAG2 monoclonal antibody (1H3L8) (Product# 702499) by western blotting. (Image courtesy of Thermo Fisher Scientific.)

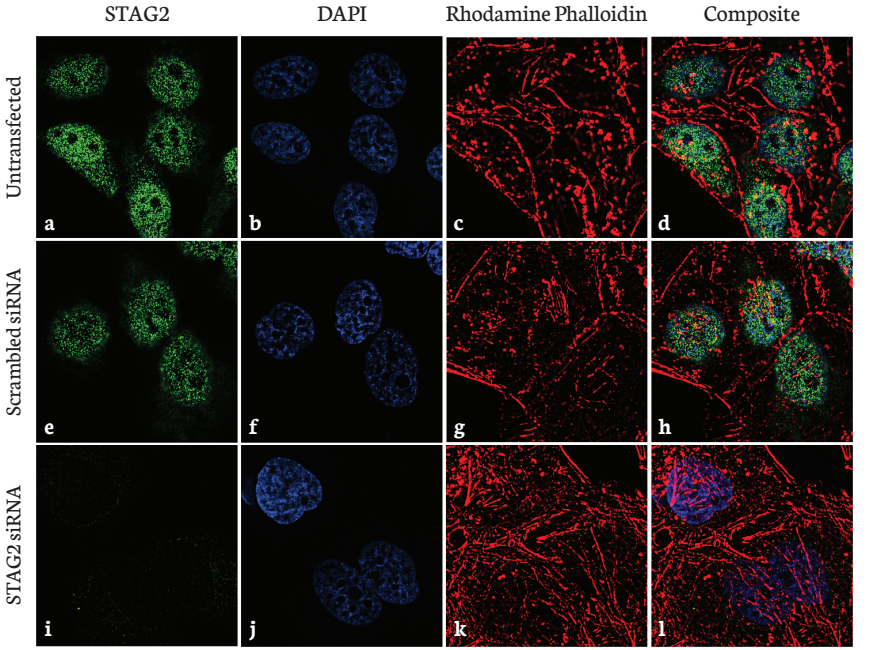

Figure 6. Detection of STAG2 by immunofluorescence in HeLa cells upon siRNA transfection using Invitrogen™ ABfinity™ STAG2 recombinant rabbit monoclonal antibody (1H3L8) (Product# 702499). (Image courtesy of Thermo Fisher Scientific.)

the nucleus and cytoplasm. During prophase, it starts to detach from the condensing chromosomes. Detachment and separation from the chromosomes is complete during metaphase, and cytokinesis, when it moves from the nucleus into the cytoplasm.

Together, the data discussed above underscore the high quality of the ABfinity STAG2 monoclonal antibody, which was generated through Thermo Fisher Scientific's proprietary recombinant monoclonal antibody generation platform across the applications tested. This antibody enables a user to study endogenous STAG2 under biologically appropriate conditions and models.

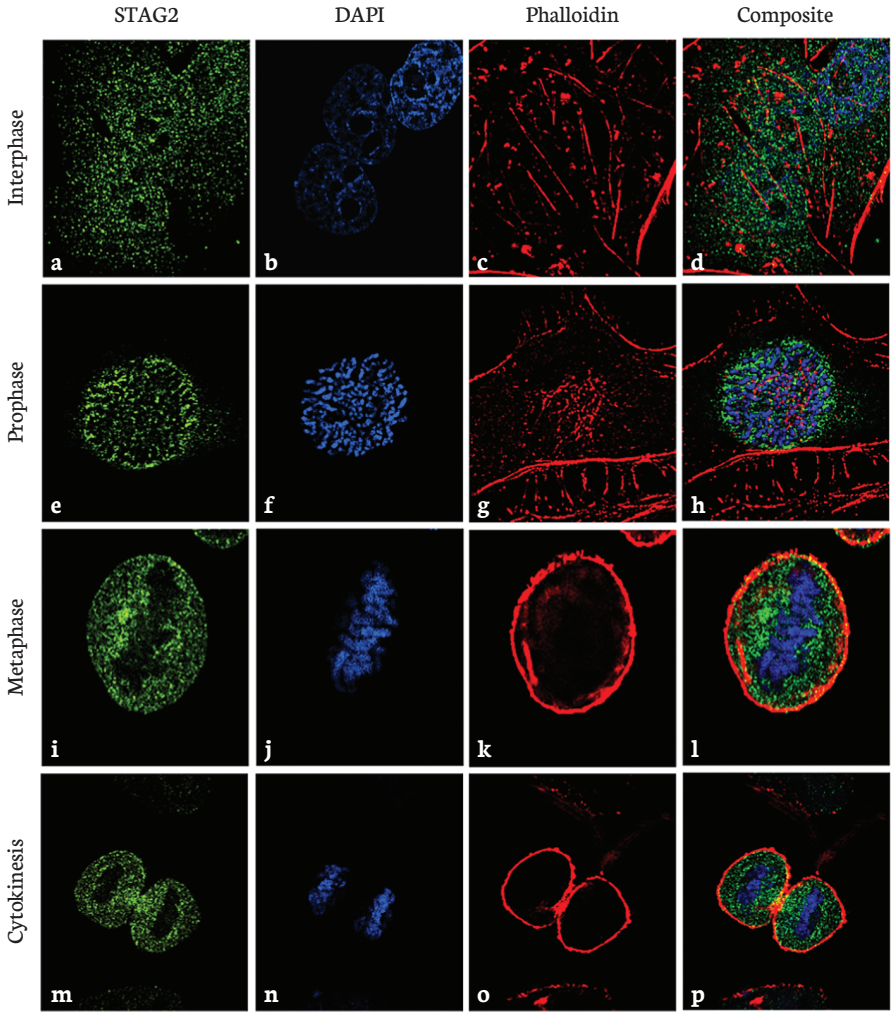

Figure 7. Detection of STAG2 in HeLa cells during different stages of the cell cycle by immunofluorescence using ABfinity STAG2 recombinant rabbit monoclonal antibody (1H3L8) (Product# 702499). (Image courtesy of Thermo Fisher Scientific.)

PUBLISHER'S PARAMETERS

To make sure that articles involving antibody-related research meet the standards of validation, publishers should request a substantial list of information. Making such antibody information available allows the journal editors and reviewers to assess the research more comprehensively before publication. It also allows other scientists to analyze the results, as well as create an opportunity to compare data from different experiments or labs. Furthermore, revealing a complete set of descriptors for an antibody used in published research makes it possible to later troubleshoot experiments that fail or to better understand why some results conflict. As new information is acquired, complete knowledge of previous uses of antibodies enhances the capability of integrating old findings with new ones.

Specifically, publishers should insist on an antibody ID, such as an antibody catalog number. This gives other scientists the necessary information to assess research results based on that antibody or using it in any way. The target of an antibody should also be reported. In addition, researchers should indicate any cross-reactivity of the antibody with other species. This must be tested in most cases, because the source and test organisms usually vary.

Publishers should also require scientists to report the source of antibodies used in articles. This includes the supplier and the lot number. How an antibody is made also helps to characterize it.

All researchers should run the necessary controls, and the results should be reported. Some antibody applications require further controls. For instance, some applications would be required to control for the effect of blocking peptides for IHC localization.

Although all of these procedures add up to a lot of testing, data collection, and reporting, this is exactly what is needed to reduce the perpetuation of false conclusions. Some journals already impose

strict requirements for research that involved antibodies. The author guidelines for the *Journal of Neuroscience Research*, for example, include a section specifically on antibody reporting. For each antibody used, authors must provide a table that includes very specific information, much of which is mentioned above, but often in even greater detail. For instance, in discussing the structure of the immunogen that an animal was immunized against, researchers must include "the exact structure," while "a vague reference to a part of the molecule is not acceptable."

To submit antibody-related research to the *Journal of Neuroscience Research*, authors must also include a section in the methods called "Antibody Characterization," which should include "a brief paragraph for each antibody used, explaining how it was characterized, and providing appropriate controls." As the author guidelines note: "Characterization includes information that assures the reader that the antibody specifically recognizes its supposed target." If that characterization involves WB, for example, the species and tissue used must be noted, and the molecular weight and pattern of the bands stained must be included. The guidelines add that the characterization might include information on ELISAs, radioimmunoassay, or other methods.

The guidelines also point out specific controls that can be used. These include attempting to stain samples from animals in which the target has been knocked out.

The purpose of the antibody, however, impacts the information required for an article in the *Journal of Neuroscience Research*. "For antibodies used as tissue markers (rather than to establish a novel and unique localization of the antigen), it is sufficient to indicate that the antibody stains the appropriate pattern of cellular morphology and distribution as demonstrated in previous publications, which should be cited."

WHAT'S NEXT?

Creating an approach to antibody validation that works for everyone in every case poses a significant challenge. Antibodies come from many suppliers and the reagents get used by even more researchers in a wide variety of ways. Moreover, the applications of antibodies continue to evolve, from novel antibodies to the evolution of methodology for existing antibodies and techniques. Most of the antibody community agrees on the need of standards, but no consensus exists for just what those should be. That is a clear goal for the near future. Despite the breadth of the antibody community, three of the central stakeholders—suppliers, publishers, and researchers—can take some clear actions to improve the reliability of tools and results.

For suppliers, increasing transparency relating to antibody production and validation is a good start. If more suppliers use a process, like the one described above, for validating antibodies, and provide a transparent account of how they obtained their results to ensure antibody validation, scientists will be able to make more thoughtful selections of antibody sources. In addition, antibody producers must ensure ongoing high quality control throughout production and ensure that customers always receive accurate and updated information, including how lots get validated.

For publishers, requirements such as the ones instituted by the *Journal of Neuroscience Research* go a long way toward ensuring accurate results from antibody-based research. Given the potential for high variability in an antibody's performance in different research scenarios, it is imperative that journals set high standards of transparency from scientists.

Such requirements also raise the bar for the researchers themselves, especially regarding the information collected and analyzed. Although pharmaceutical and large biotechnology companies often dedicate

large groups of scientists to validating antibodies, academic labs do not have that luxury. Nonetheless, these labs must meet similar levels of antibody validation. In large part, academic labs must rely on information from antibody producers, but scientists must also run all of the necessary controls and characterize the performance of an antibody in specific-use cases. This means that all principle investigators must train lab personnel to run the necessary tests and collect the results that are needed.

Clearly, only coordinated teamwork among all stakeholders can address the problem of antibody validation. Although considerable progress has been made, much work lies ahead. Until the validation of antibodies can be trusted from every supplier, all scientific research and each article that mentions an antibody, the conclusions from such work will remain suspect. With such antibody validation standards in place, however, the scientific progress will move ahead much more effectively.

FURTHER READING

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