

Proposal for first validating antibody specificity strategies published

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The International Working Group on Antibody Validation (IWGAV), an independent group of international scientists with diverse research interests in the field of protein biology, today announced the publication of initial strategies developed to address a critical unmet need for antibody specificity, functionality and reproducibility in the online issue of *Nature Methods*. The IWGAV is the first initiative of its size and scope to establish strategic recommendations for antibody validation for both antibody producers and users.

Although antibodies are among the most frequently used tools in scientific research, there is not a comprehensive scientific framework for the validation of antibody specificity across research applications. The use of poorly characterized antibodies has been estimated to cost \$350 million each year in the United States alone and \$800 million worldwide as a result of failed or unreliable experiments.

"This publication is an important first step towards the development of widely accepted standards for validating antibodies and ensuring high quality and consistent antibodies for biomedical research," said Dr. Mathias Uhlen, professor of microbiology, Royal Institute of Technology, Stockholm, Sweden and chair of the IWGAV. "We look forward to receiving feedback from the broader community of antibody users, publishers, funding agencies and producers to help strengthen this initial proposal and ensure the reliability of these essential tools of biomedical research."

The IWGAV identified five "conceptual pillars" to guide antibody validation in specific research applications:

- **Genetic strategies:** Measure the relevant signal in control cells or tissues in which the target gene has been knocked out or knocked down using techniques such as CRISPR/Cas or RNAi.
- **Orthogonal strategies:** Use an antibody-independent method for quantification across multitudes of samples and then examine the correlation between the antibody-based and antibody-independent quantifications.
- **Independent antibody strategies:** Use two or more independent [antibodies](#) that recognize different epitopes on the target protein and confirm specificity via comparative and quantitative analyses.
- **Expression of tagged proteins:** Modify the endogenous target gene to add sequences for an affinity tag or a fluorescent protein. The signal from the tagged protein can be correlated with detection through antibody-based methods.
- **Immunocapture followed by mass spectrometry (MS):** Couple immunocapture, the technique of isolating a protein from a solution through binding with a target-specific antibody, with MS analysis to identify proteins that interact directly with the purified antibody as well as proteins that may form a complex with the [target protein](#).

While each of these conceptual pillars may provide evidence of antibody specificity, the IWGAV recommends multiple pillars be used to claim a particular antibody has been well validated for use in a specific application. The manuscript in *Nature Methods* also includes recommendations for producers and users to ensure antibody reproducibility over time.

The proposal, published in *Nature Methods* is intended to provide a

scientific foundation for antibody validation and reproducibility standards that encourage the widespread adoption of comprehensive guidelines for antibody use. However, this will require continued input from the global research community and antibody producers. Each of these stakeholders will have important roles to play in the adoption and implementation of this proposal.

More information: A proposal for validation of antibodies, *Nature Methods*, [nature.com/articles/doi:10.1038/nmeth.3995](https://doi.org/10.1038/nmeth.3995)

Thermo Fisher Scientific, the world leader in serving science, provided financial support to the IWGAV in 2015 to spearhead the development of industry standards and help combat the common challenges associated with antibody specificity and reproducibility.

Provided by The International Working Group on Antibody Validation (IWGAV)

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