HOW TO TARGET VALIDATE YOUR ANTIBODY

MAKE SURE IT WORKS IN YOUR APPLICATION!

THE GUIDELINES

Due to the need for properly validated antibodies, the International Working Group on Antibody Validation (IWGAV) has made an effort to standardize best practices, resulting in a publication proposing "Conceptual Pillars for Validation of Antibodies." The pillars presented here are directed to both users and producers of antibodies.

ANTIBODYPEDIA

The Antibodypedia database lists antibodies provided by academia and commercial companies. Antibodypedia ranks antibodies based on the amount and quality of the knowledge associated with them, putting the antibody with the most information available on top of the search list, and assisting you in selecting the most appropriate antibody for your experiment.

GENETIC STRATEGIES

Genetic strategies can be used to generate genetically modified samples where the target protein is knocked out or knocked down. This method provides a direct link between the gene and the target protein. The antibody is considered validated for its target when the signal from the original sample is significantly downregulated in the genetically modified sample.

ORTHOGONAL STRATEGIES

Orthogonal strategies compare an antibody-based method with an antibody-independent method, for example targeted proteomics approaches using labeled internal standards. Identifying and measuring your target protein in a set of samples with a method not involving antibodies should give comparable results to the antibody-based method.

INDEPENDENT ANTIBODIES

Independent antibody strategies use two or more antibodies recognizing different epitopes (binding sites) on the target protein. This method minimizes the likelihood of off-target binding to the same unrelated protein. Antibody validation is achieved when the unique antibodies give comparable results when using the same detection method.

TAGGED PROTEINS

Tagging proteins on the genetic level with an affinity tag or a fluorescent protein can be used to validate the antibody for the target protein. Tagged proteins should preferably be expressed at endogenous levels. The expression pattern of the tag should overlap with the expression pattern created when using the antibody for protein detection.

IMMUNO-CAPTURE MS

Immunocapture is a method that uses an antibody to isolate a protein from a solution. When coupling this technique with mass spectrometry (MS), the proteins captured by the antibody can be identified. The peptides for the target protein should be on the top of the generated peptide list in order for the antibody to be considered specific.

FIVE CONCEPTUAL PILLARS FOR ANTIBODY VALIDATION. Antibodies are powerful tools used in many different applications to detect proteins. The power of the antibody lies in its ability to recognize a specific target. It is crucial to properly validate the antibody for binding to its intended target, to test the antibody in the intended application, and to understand the context where it will be used. The five pillars for antibody validation are summarized below.

	PILLAR				
Application	Genetic Strategies	Orthogonal Strategies	Independent Antibodies	Tagged Proteins	Immunocapture Mass Spectrometry
Western blotting (WB)	♦	♦	♦	♦	
Immunohistochemistry (IHC)	♦	♦	♦	♦	
Immunocytochemistry (ICC)	♦	♦	♦	♦	
Flow sorting (FS)	♦	♦	♦	♦	
Sandwich assays (SA)	♦	♦	♦		
Immunoprecipitation (IP)	♦		♦		♦
Reverse phase protein arrays (RPPA)	♦	♦	♦		

This table summarizes for which applications the five conceptual pillars are recommended. The ♦ represents support for the pillar in the application. Ref.: M. Uhlen *et al.*, A proposal for validation of antibodies. *Nat. Methods* **13**, 823–827 (2016).

Other	FS	ICC	IHC	WB
5%	6%	9%	29%	51%

Percentage utilization of antibodies in listed applications according to data in Antibodypedia. Western blotting (WB) is the most commonly used application, followed by immunohistochemistry (IHC), immunocytochemistry (ICC), and flow sorting (FS).

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