alidated antibodies, the Internationa **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.

#### **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.

#### **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.

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### **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

#### **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.



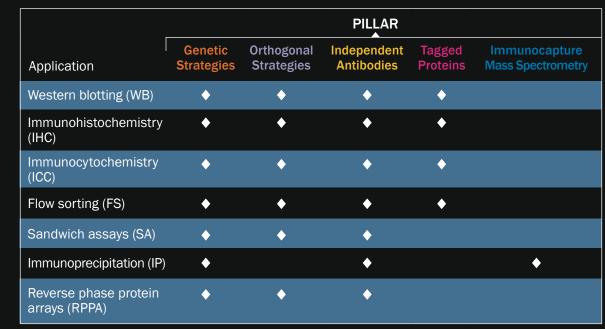
Immunocapture is a method that uses an antibody to isolate a protein from a solution. When coupling should be on the top of the generated peptide list in order for the antibody

## **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.

# **CAPTURE MS**

this technique with mass spectrometry (MS), the proteins captured by the antibody can be identified. The peptides for the target protein to be considered specific.



HOW TO TARGET VALIDATE

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

understand the context where it will be used. The five pillars for antibody validation are summarized below.

properly validate the antibody for binding to its intended target, to test the antibody in the intended application, and to

YOUR ANTIBODY

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).







