Validateyour Antibodies

Antibodies are among the most common reagents in both research and clinical laboratories for

WB	WESTERN BLOTTING
IHC	IMMUNOHISTOCHEMISTRY
ICC	IMMUNOCYTOCHEMISTRY

QUANTITATIVE IMMUNOFLUORESCENCE

ENZYME-LINKED IMMUNOSORBENT ASSAYS

IMMUNOPRECIPITATION

CHROMATIN IMMUNOPRECIPITATION

FLOW CYTOMETRY

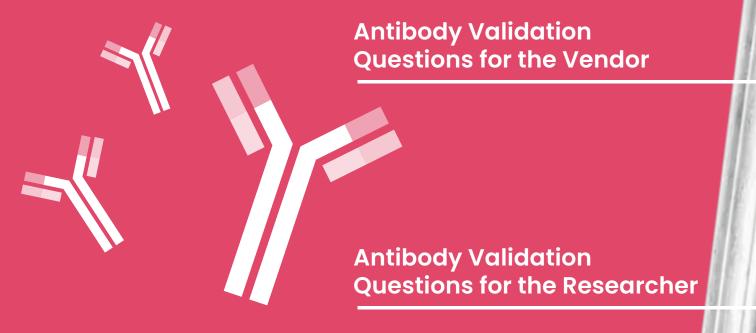
It is estimated that there are more than 300 antibody companies that sell over 2 million antibodies for the research and clinical markets (www.antibodyresource.com/onlinecomp.html, www.citeab.com).

When it comes to research use, there are no standard guidelines in place for manufacturing, validating, and using antibodies.

Pitfalls of not validating your antibodies

- Incorrect, misleading data
- Irreproducibility





When purchasing an antibody, do not depend solely on:

- The vendor's word
- Western blot we evidence claiming a single band migrating at the predicted molecular weight



The ultimate responsibility for the validity of the antibody lies with you, the purchaser, not the vendor!

The MOST IMPORTANT QUESTION to ask yourself:

Does the antibody recognize its intended target in my assay?

ASK YOUR VENDOR

- Is a data sheet supplied describing the antibody and its intended target protein, immunogen source, animal host, recommended applications, recommended starting dilutions and buffers, relevant protocols and references?
- Are data supplied for recommended or qualified applications, and is there a description of the methods used to validate the antibody for those applications?
- Are recommendations given on the proper use of positive and negative controls? - Has the antibody been tested against endogenous protein?
- Is the antibody made and tested in-house by the vendor or by a third party? - Are references and citations provided to corroborate stated claims?
- Is technical support offered for this product?

ASK YOURSELF

- Has the antibody been shown to react with my species of interest?
- In cases where the epitope is defined, is the epitope conserved with the protein in my species of interest?
- Can I use my own lab protocols or must I use those recommended by the vendor?
- Have I included appropriate positive controls (nonexpressing cells transfected with the protein of interest, protein-overexpressing cells, cells treated with target the protein, knockout cells, sirna or shrina knockdown controls, cells treated the protein, knockout cells, siRNA or shRNA knockdown controls, cells treated with target inhibitors)?
- Should I aliquot the antibody?
- What is the shelf-life of the antibody?
- How should I store the antibody?
- Have I kept track of all the relevant information pertaining to the antibody such as vendor name, lot number, product number, expiration date and such?
- Is the antibody from the same lot that I used previously? Have the recommended dilutions changed? Does it perform similarly to the previous lot in my assay?

Varying degrees of validation can be applied depending on the application in which the antibody will be used.

For example, a clinically geared immunohistochemistry assay 😁 will require a high degree of antibody validation at multiple levels:

- A single band detected in western blots of sample lysates or immunoprecipitations of states. immunoprecipitations 🕟 at the expected molecular weight.
- The single band in WB 🍩 and the signal in immunofluorescence assay is diminished by RNAi or absent in negative tissue or cell lines.
- Staining is localized, specific, and consistent with the literature.
- The antibody results are reproducible between lots, runs, and personnel.

Recommended methods and controls to determine if an antibody is recognizing its intended target

