Validate your Antibodies

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

Did you Know?

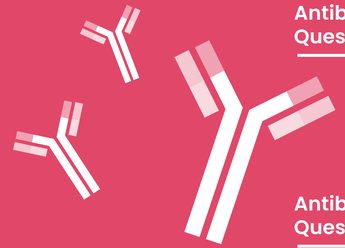
- WB — WESTERN BLOTTING
- IHC — IMMUNOHISTOCHEMISTRY
- ICC — IMMUNOCYTOCHEMISTRY
- QIF — QUANTITATIVE IMMUNOFLUORESCENCE
- ELISA — ENZYME-LINKED IMMUNOSORBENT ASSAYS
- IP — IMMUNOPRECIPITATION
- ChIP — CHROMATIN IMMUNOPRECIPITATION
- FC — FLOW CYTOMETRY

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.

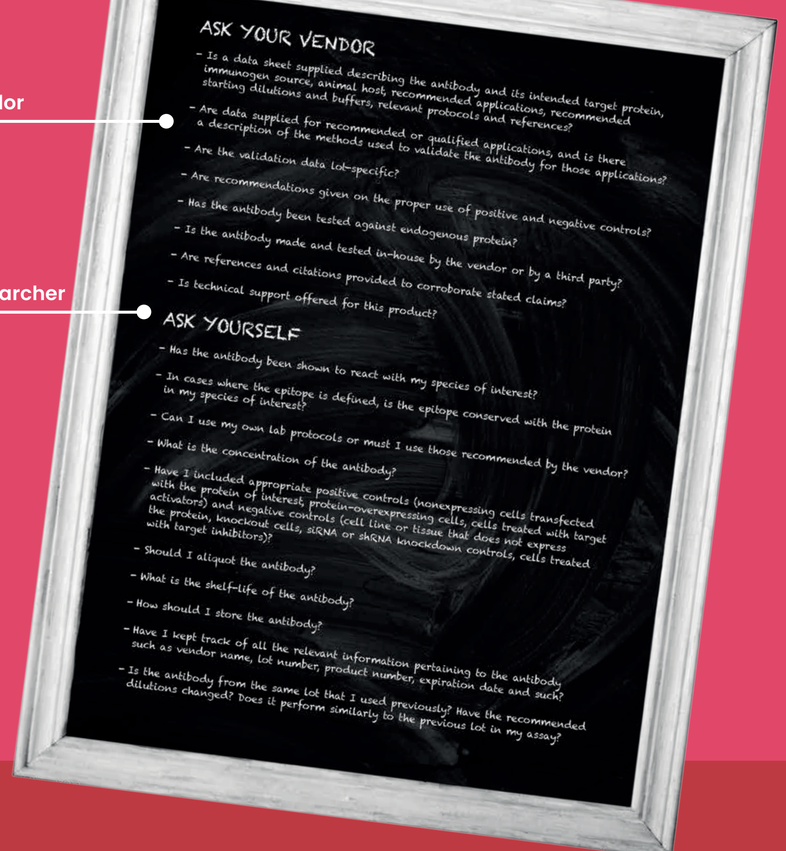
Pitfalls of not validating your antibodies

- Incorrect, misleading data
- Irreproducibility



Antibody Validation Questions for the Vendor

Antibody Validation Questions for the Researcher



When purchasing an antibody, do not depend *solely* on:

- The vendor's word
- Western blot (WB) 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?

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 (IHC) will require a high degree of antibody validation at multiple levels:

- A single band detected in western blots (WB) of sample lysates or immunoprecipitations (IP) at the expected molecular weight.
- The single band in WB (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

	WB	IHC	ICC	ELISA	IP	ChIP	FC
Is detection reduced in samples after siRNA knockdown?	●		●	●	●	○	●
Is detection absent in samples from knock out tissue?	●	●		●	●	○	●
Is detection absent in naturally negative cell lines or tissues?	●	●	●	●	●	○	●
Do two or more antibodies against disparate epitopes reciprocally identify the target in western blot of IPs?	●				●		
Can expression level be correlated in another type of assay (e.g., enzyme activity, WB, IP, ELISA)?	●	●	●	●	●	○	●
Do two or more antibodies against disparate epitopes show relatively similar patterns?	●	●	●	●	●	○	●
Is the subcellular localization in agreement with the literature?		●	●				
Does the use of protein activators or inhibitor smodify the detection of post translational modifications?	●		●	●	●	○	●
Does expression and detection of epitope-tagged protein agree with results of studies of the endogenous protein?	●		●	●	●	○	
Is the signal from an isotype control low to negative?		●	●	●	●	○	●
Are the results reproducible between runs, lots, personnel?	●	●	●	●	●	○	●