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

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

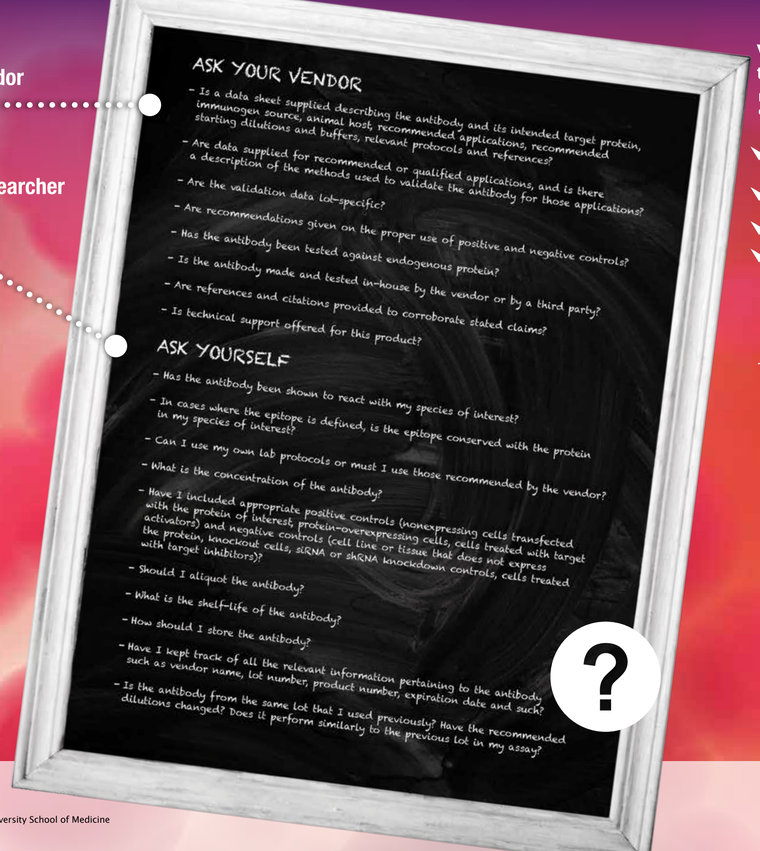
The ultimate responsibility for the validity of the antibody lies with you, the purchaser, not the vendor!

The MOST IMPORTANT QUESTION to ask yourself:

Does the antibody recognize its intended target in my assay?

Antibody Validation Questions for the Vendor

Antibody Validation Questions for the Researcher



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 knockout 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 inhibitors modify the detection of posttranslational 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?	●	●	●	●	●	●	●

Project Advisors

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Sponsored by: BETHYL LABORATORIES, INC. Really Good Antibodies

Bethyl Laboratories, Inc. has been dedicated to supporting scientific discovery through its qualified antibody products and custom antibody services since its founding in 1972.

Currently, Bethyl's portfolio consists of over 7,150 catalog products: close to 5,700 primary antibodies targeting over 2,700 proteins and 1,450 secondary antibodies raised against immunoglobulins from over 25 species.

With over 40 years of experience, Bethyl is also a leading provider of custom antibody production services. Bethyl offers complete packages from initial peptide synthesis to affinity purification of custom antibodies from an antigen-specific immunosorbent.

Every antibody that Bethyl sells has been manufactured to exacting standards at its sole location in Montgomery, Texas, and has been validated in-house by Bethyl's team of scientists. From the veterinary facilities to the development, production and validation labs, the entire Bethyl team focuses on delivering quality products.

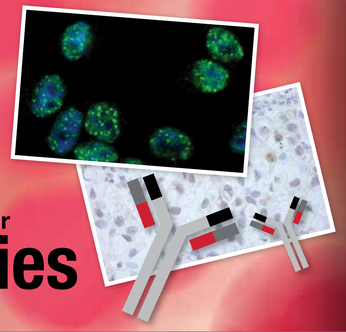
What does it take for antibody to pass Bethyl's validation and quality control? In other words, what makes a really good antibody?

First and foremost, an antibody must be shown to recognize the intended protein target. An antibody must also show high sensitivity with minimal cross-reactivity. To achieve these goals, Bethyl has devised a unique process for validating the specificity of its antibody products. While many companies settle on using data from a single antibody, Bethyl utilizes a reciprocal testing system that incorporates multiple antibodies raised to different regions of the same protein target.

Only after an antibody has been validated for specificity in immunoprecipitation and/or Western blot is it then tested in additional applications such as immunohistochemistry, immunocytochemistry, ChIP, and proximity ligation assay.

Because of its rigorous validation process and high standards, Bethyl does not sell every antibody it makes. Bethyl serves to advance science by concentrating its resources on developing qualified antibodies, including many to emerging and underserved protein targets.

***To learn more about Bethyl's validation process and its products, please visit* www.bethyl.com.**



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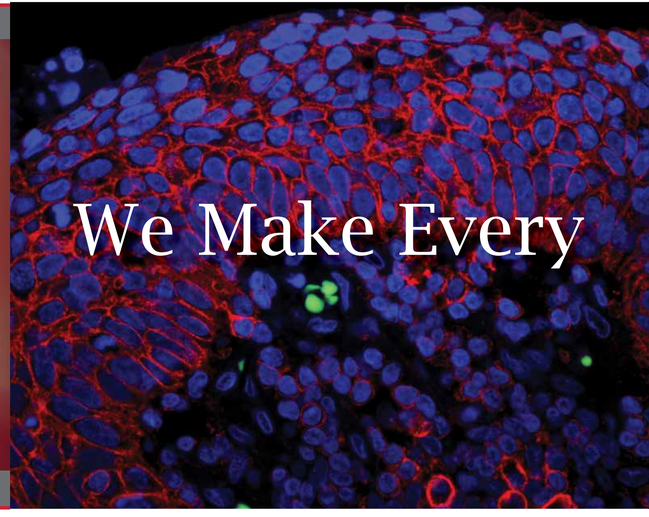

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