

abcam

# Raising antibody standards

Providing life scientists with specific and consistent antibodies

2016

## Why is quality an issue?

We're currently facing a problem with reproducibility in science: 70% of scientists have failed to reproduce another scientist's experiments, and over 50% have failed to reproduce experiments of their own design<sup>1</sup>. With so much research often built on previous work, this is a major issue; consider how much further we could push scientific discovery if we weren't limited by shortcomings in reproducibility.

One part of this complex problem is reagent quality, specificity, and consistency. Since reagents like antibodies are an integral part of all research, we need to take steps to improve their quality where we can.

Problems with antibodies have recently been profiled across numerous media channels, and attention has been drawn to a number of landmark studies that highlight the research and financial costs incurred<sup>2-7</sup>. An analysis in PLoS Biology, for example, suggests that over half of preclinical research is irreproducible, equating to approximately US \$28 billion spent every year on work that cannot be replicated<sup>8</sup>. The analysis establishes four categories of preclinical irreproducibility, with biological reagents and reference materials being the top offender – accounting for 36.1% of total irreproducible research.

Even when high-quality reagents are used, lack of reproducibility is further exacerbated by poor reporting of methods. One study found that only 44% of publications included enough information about the antibody, such as the supplier and clone, to allow other researchers to purchase the same product and reproduce the experiments<sup>9</sup>.

In an effort to remedy this situation, both researchers and suppliers are looking at ways they can address these issues of quality and reproducibility. It's not an easy task to drive change, but it's the right direction to take in order to further science faster.

## Antibody validation methods

Antibody validation is a means of demonstrating that an antibody performs, in terms of specificity and reproducibility, as intended and is fit for purpose. There are a variety of experimental methods available to achieve this, each with their own set of distinct benefits and limitations. Some techniques, like gel shift assays, western blotting, immunoprecipitation (IP), ELISAs, immunohistochemistry (IHC), and immunocytochemistry (ICC) have been used for a number of years and continue to be important tools. More recently, advanced techniques, like knockout (KO), knockdown, peptide array, and mass spectrometry, have become increasingly popular.

It is important to note that there is no single solution to antibody validation and quite often a combination of techniques is the best approach.

**Table 1.** The benefits and limitations of several key antibody validation methods.

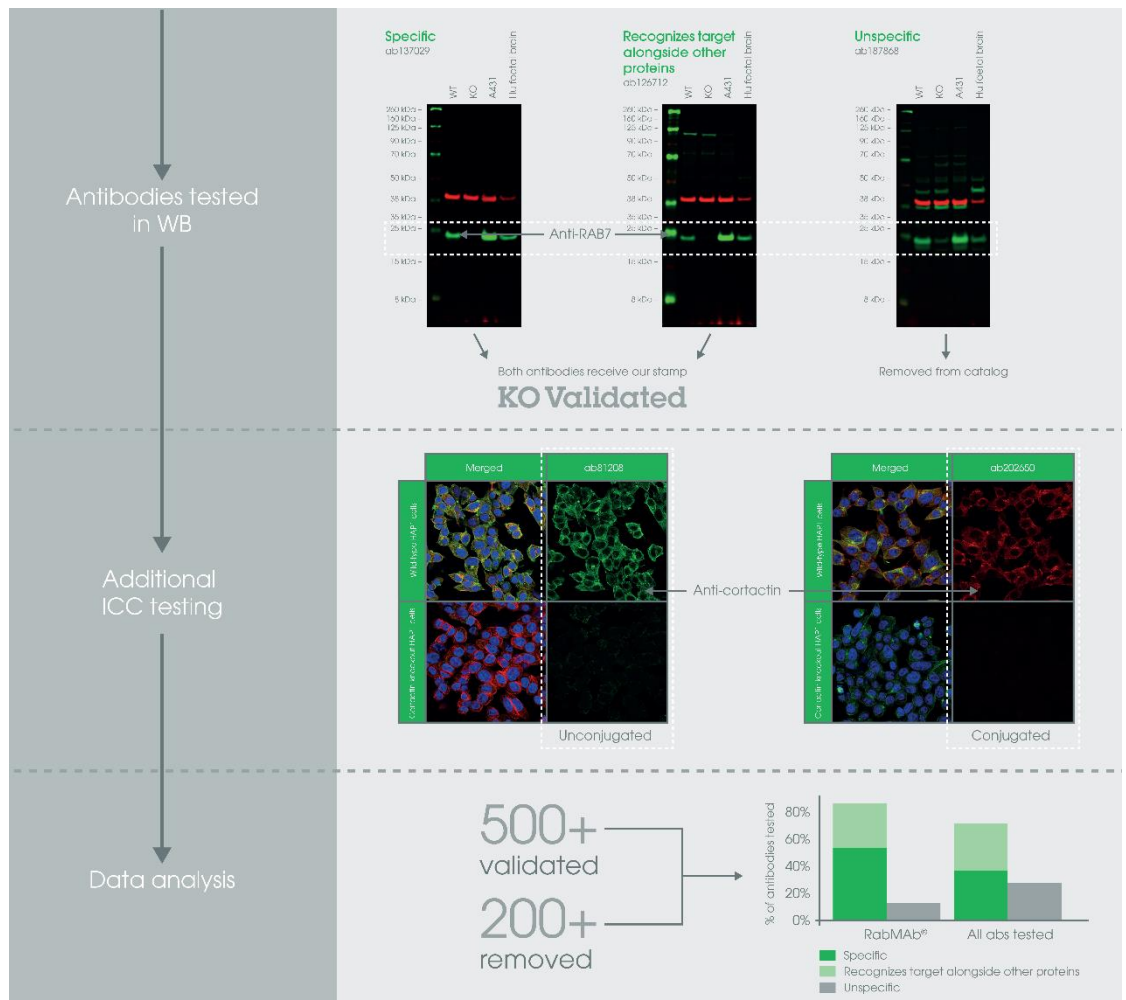
Validation	Benefits	Limitations
<b>KO models</b>	<ul style="list-style-type: none"> <li>+ KO cell lines function as a true negative control</li> <li>+ Guaranteed no expression of target gene</li> <li>+ A potentially large number of KO cell lines may be generated in a short period of time</li> <li>+ Knockout cell lines may be used in all assays - western blot, IHC, ICC, flow cytometry</li> </ul>	<ul style="list-style-type: none"> <li>- Knockout cell lines against specific genes are not always viable</li> </ul>
<b>Mass spectrometry/ IP-MS</b>	<ul style="list-style-type: none"> <li>+ Confirms specificity based upon digested protein fragments</li> <li>+ Amenable to a high throughput format</li> <li>+ Potential to estimate abundance of target protein bound to the antibody of interest using normalization techniques</li> </ul>	<ul style="list-style-type: none"> <li>- IP-MS assays difficult to optimize</li> <li>- Specialized technique that requires use of a mass spectrometer</li> <li>- For some targets, protein binding to the antibody of interest via IP is ineffective</li> <li>- Can be difficult to distinguish partner proteins pulled down in a complex from off-target binding</li> </ul>
<b>Western blotting</b>	<ul style="list-style-type: none"> <li>+ Useful for determining antibody specificity against target protein based upon molecular weight</li> <li>+ Ideal for detecting either native or denatured proteins</li> <li>+ Qualitative assay</li> </ul>	<ul style="list-style-type: none"> <li>- Time-consuming assay</li> <li>- Difficult to determine the optimal experimental conditions (ie methodology and buffer)</li> <li>- Only a small number of antibodies may be validated per run</li> </ul>
<b>IHC and ICC</b>	<ul style="list-style-type: none"> <li>+ Validates whether an antibody recognizes the correct protein based on cellular localization</li> <li>+ Specificity confirmed based upon cells that either do or do not express the target protein</li> <li>+ Qualitative assay</li> </ul>	<ul style="list-style-type: none"> <li>- Unable to determine if an antibody recognizes other proteins non-specifically with identical cellular localization</li> <li>- Often difficult to determine cell or tissue types that either do or do not target the protein</li> </ul>
<b>Protein/ peptide array</b>	<ul style="list-style-type: none"> <li>+ Allows screening of a larger number of over-expressed proteins</li> <li>+ Very high-throughput screening process</li> <li>+ Requires very small sample volumes</li> </ul>	<ul style="list-style-type: none"> <li>- Protein array only: unable to screen for post-translationally modified proteins</li> <li>- Only present linear epitopes for interrogation – do not usually present conformational epitopes</li> </ul>
<b>siRNA knockdown</b>	<ul style="list-style-type: none"> <li>+ Confirms specificity through target protein being downregulated</li> <li>+ Knockdown cells lines may be used in all assays – western blot, IHC, ICC, flow cytometry</li> </ul>	<ul style="list-style-type: none"> <li>- Knockdown is transient</li> <li>- Difficult experiment to optimize – often requiring several siRNA sequences</li> </ul>

# Leading the charge on antibody quality

As a leading supplier of reagents for life scientists, we have taken it upon ourselves to raise industry qualities. We are doing this through four main approaches:

## 1. Confirming antibody specificity

We are addressing specificity issues with an ongoing KO-validation program using human KO cell lines generated from haploid cellular models via CRISPR/Cas9. In the context of antibody quality, KO models provide an excellent standard for antibody validation as they represent a true negative control.



**Figure 1.** Our knockout (KO) validation process. **Top:** antibodies tested in western blot (WB). Results are graded as “Specific” (signal in the wild-type (WT) and not the KO samples), “Recognizes target alongside other proteins” (signal in the WT but not the KO samples at the region of interest, while signal observed in both samples at various molecular weights), or “Unspecific” (zero or limited reduction of signal in the KO compared to the WT sample at the region of interest). **Middle:** Immunocytochemistry (ICC) testing carried out when deemed important to an antibody or protein target. **Bottom:** analysis of KO testing data resulted in over 500 antibodies being validated to recognize their target protein, more than 200 antibodies being deemed unspecific and subsequently removed from our catalog, and showed our RabMAb® range to be more specific than other monoclonal or polyclonal antibodies.

Genetic KO models are so powerful since they allow scientists to understand the function of a particular gene by observing the loss-of-function phenotype in whole animal or cellular models. Over the past year, we have worked with Horizon Discovery to KO validate over 500 antibodies and removed a further 200 unspecific antibodies from our catalog (Figure 1). KO validation is just one of many steps we are taking to raise antibody standards.

## 2. Setting catalog and supplier standards

While KO is an exceptionally powerful control, it is still an indirect method to determine antibody specificity. For this reason, we know there is a need to use multiple validation methods to support KO studies. This is why in addition to KO validation, we carry out QC checks via ICC/IF, IHC, flow cytometry, ELISA, IP, chromatin IP (ChIP), and peptide array to make sure that all of our products achieve the required levels of activity, stability, and performance.

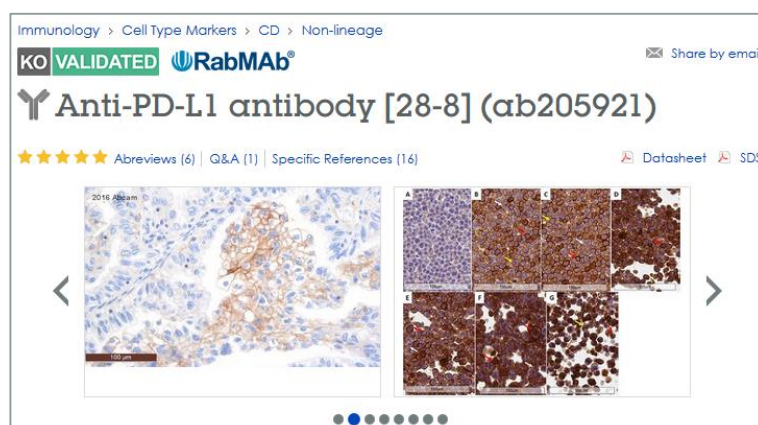
Keeping our catalog standards high, means holding our supplier to the same standards we use internally. At present, we work closely with over 400 trusted suppliers to ensure that they deliver products of a high enough quality. All product data we receive from suppliers is reviewed thoroughly to check that the product works as intended or is specific for the target protein. This data is scored using an image grading system generated from extensive in-house characterization data. Only products that pass this grading process are added to our catalog.

We also proactively test many of these on an ongoing basis and follow up on feedback from customers. When any issues come to light, we subject those products to additional testing. If they fail our internal specifications, they're removed from our catalog and customers are contacted as soon as possible. Similarly, when suppliers fail to meet our standards, we stop working with them.

## 3. Data availability and feedback


To ensure that you have all relevant information about a product, our datasheets include extensive data around the applications the product has been tested in. They also contain practical information like the dilution and storage recommendations, immunogen information, and species reactivity.

We have an open review policy, known as Abreviews, whereby researchers can post positive and negative feedback about a product. If you've ever used customer reviews, you'll know how valuable they can be.



**Figure 2.** A catalog entry for anti-PD-L1 [28-8] (ab205921) showing IHC data and linking through to Abreviews from customers, any relevant questions, and the list of peer-reviewed articles that use and cite this antibody.

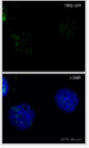

Our Abreviews program allows you to help other scientists by sharing your knowledge on how our products worked in your application and species (Figure 3). This up-to-date information often provides useful data about new applications, optimal dilution conditions, and images of our products at work. This also helps us remain aware of possible problems, at which point we initiate further testing to maintain quality standards.



### Immunocytochemistry/ Immunofluorescence abreview for Anti-GFP antibody - ChIP Grade

★★★★★ Excellent

**Abcam guarantees this product to work in the species/application used in this Abreview.**

<b>Application</b>	Immunocytochemistry/ Immunofluorescence	
<b>Sample</b>	Human Cell (U2OS)	
<b>Permeabilization</b>	Yes - NP40	
<b>Specification</b>	U2OS	
<b>Blocking step</b>	BSA as blocking agent for 1 hour(s) and 0 minute(s) · Concentration: 3% · Temperature: 21°C	
<b>Fixative</b>	Formaldehyde	

[Read More](#)

**Abcam user community**  
Verified customer

Submitted Feb 02 2016

**Figure 3.** An example an Abreview submitted by one of our customers using our anti-GFP (ab290) antibody.

#### 4. Pioneering new technologies

As manufacturing technology advances, so do we. To overcome the limited number of antibodies that can be generated using mouse B cells, we developed a unique method of monoclonal antibody development using rabbits: our RabMAb® platform. RabMAb antibodies combine the best properties of monoclonal antibodies with the most desirable attributes of rabbit polyclonal antibodies: diverse epitope recognition, high affinity and specificity, and cross-species reactivity.

In order to promote consistency alongside sensitivity and specificity, we have embraced recombinant technology. In fact, the vast majority of RabMAb antibodies we offer are recombinant, and therefore avoids hybridoma cell drift and batch-to-batch variation since the production processes are controlled and reliable. Our recent incorporation of Axiomx's phage display-based *in vitro* antibody technology complements the RabMAb platform and allows us to undertake the reproducible production of new monoclonal antibodies in just weeks, rather than months.

We've been driving the shift towards better antibodies and today, we have over 9,000 recombinant monoclonal RabMAb antibodies, provide custom RabMAb services to over 700 universities, institutes, and companies globally, and have developed more than 275 IVD-grade IHC antibody clones.

## Improving antibody quality is a joint effort

While suppliers are central to improving antibody quality, there are additional changes that can be made by both researchers and publishers to help tackle irreproducibility in science. Reagent suppliers need to undertake appropriate application-specific validation of reagents; researchers to implement good reporting practices; and publishers and funding agencies could also insist that reagents used or proposed for use have been sufficiently validated and reported to further promote reproducibility.

### What suppliers can do

Antibody suppliers provide the bulk of antibodies in experimental use. It is, therefore, vital that we adopt best practices and are open about the methods used when characterizing antibodies.

- **Manufacturing practices:** in addition to manufacturing at the highest standards, suppliers need to employ the best available techniques, like advanced immunogen design and recombinant technology.
- **Validation:** suppliers need to make use of the most appropriate available methods to validate their antibodies. This gives researchers the confidence that their antibody will generate data that are accurate and precise.
- **Data:** all of the data associated with an antibody needs to be freely available so that end researchers have the relevant information to make informed decisions when either purchasing or troubleshooting.

### What researchers can do

Researchers have an important part to play in promoting reproducibility through robust experimental design, trusted protocols, and accurate reporting.

- **Validation:** it is important researchers validate an antibody using their particular experimental setup. While the antibody should have been validated by the supplier, it can be difficult to account for custom solutions or protocols used by the researcher.
- **Experimental design:** the value of good experimental design cannot be underestimated. Tissue and reagent controls – positive and negative – are needed to not only account for experimental variables but to identify sources of error.
- **Detailed reporting:** when it comes to sharing results through publication, researchers are responsible for including sufficient information regarding the methods and materials used so that others can successfully reproduce the experiment.

### What publishers and funding bodies can do

Journal publishers and funding bodies can support reproducibility by asking for detailed information, such as catalog numbers and validation standards for reagents like antibodies, as part of the default submission process.



## The way forward

Improving the reproducibility of research across the life sciences needs to be a cooperative effort between all parties involved: the suppliers, the researchers, and the publishers. Suppliers need to go to ever greater lengths to ensure their antibodies are sufficiently validated and that these testing data are readily available. This first step is the most vital as it allows researchers to reproduce their own research, confident that their antibody's specificity has been confirmed long before they begin their experiments.

It is also important that suppliers include data around antibody characterization. This empowers researchers to use these data to design better experiments and report their results, methods, and materials in sufficient detail when publishing. This process can be reinforced if publishers collectively impose more stringent guidelines around reagent reporting and validation.

While issues of quality have been gathering attention, we have been leading and shaping the industry's concept of high-quality reagents: with thousands of validated antibodies, tens of thousands of scientific discussions through scientific support and Abreviews, and a KO validation initiative carried out on an unmatched scale.

We know how important your research is to you, which is why we take every measure to make sure it is as reproducible and robust as possible. To this end, we are actively contributing to discussions with the Global Biological Standards Institute (GBSI) to help define and set antibody validation standards.

Through our commitment to quality, we are striving to minimize the time, effort, and money wasted in research. By providing researchers with reagents that work first time, we are helping to speed up the progress of scientific discovery.



## References

1. Baker, M. 1,500 scientists lift the lid on reproducibility. *Nature* **533**, 452–454 (2016).
2. Baker, M. Reproducibility crisis: Blame it on the antibodies. *Nature* **521**, 274–276 (2015).
3. Baker, M. Antibody anarchy: A call to order. *Nature* **527**, 545–551 (2015).
4. Uhlen, M. *et al.* A proposal for validation of antibodies. *Nat. Methods* (2016). doi:10.1038/nmeth.3995
5. Bradbury, A. & Pluckthun, A. Reproducibility: Standardize antibodies used in research. *Nature* **518**, 27–29 (2015).
6. Begley, C. G. & Ellis, L. M. Drug development: Raise standards for preclinical cancer research. *Nature* **483**, 531–3 (2012).
7. Berglund, L. *et al.* A gene-centric Human Protein Atlas for expression profiles based on antibodies. *Mol. Cell. Proteomics* **7**, 2019–27 (2008).
8. Freedman, L. P., Cockburn, I. M. & Simcoe, T. S. The Economics of Reproducibility in Preclinical Research. *PLOS Biol.* **13**, e1002165 (2015).
9. Vasilevsky, N. A. *et al.* On the reproducibility of science: unique identification of research resources in the biomedical literature. *PeerJ* **1**, e148 (2013).