

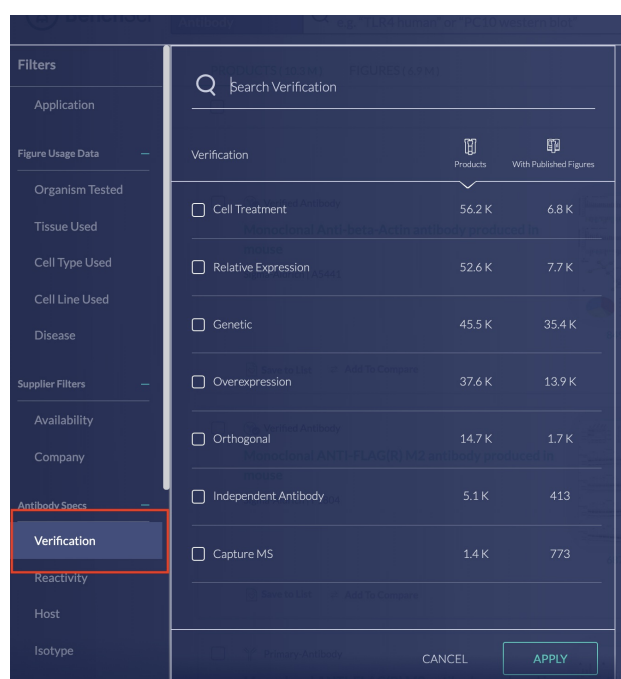
Finding verified antibodies

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The verification filter returns antibodies that have been validated by complementary experiments, as well as figures in which these verified antibodies were used

Antibody validation information is invaluable in increasing your confidence that an antibody recognizes the intended target during your antibody search process. The **Verification** filter empowers you to find antibodies whose specificity is supported by one of the [five pillars of antibody validation](#) [1], in addition to two other pillars adopted by industry-leading vendors.

The **Verification** filter empowers you to find antibodies whose effectiveness is supported by complementary experiments and see how they have been used in the literature.



Five validation pillars:

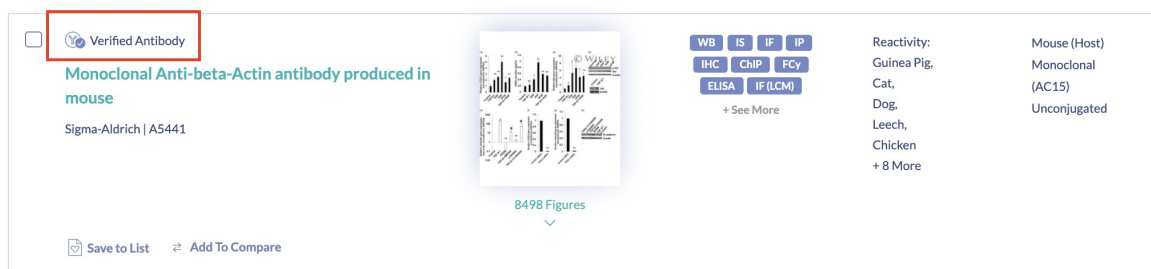
- Genetic (knockout/knockdown)*
- Overexpression*
- Orthogonal‡
- Independent antibody‡
- Capture mass spectrometry‡

Additional vendor pillars:

- Cell treatment‡
- Relative expression‡

Selecting one of these **Verification** filters in the *Antibody Specs* group of filters will show you products — or figures featuring products — that are associated with at least one verification image.

For pillars marked with (*), verification images, and data may come from vendors, third-party sources, or publications. Pillars marked with the double dagger (‡) integrate verification data from vendors and third-party sources. Verified products feature the *Verified Antibody* tag on the products results page and on figure thumbnails to give you greater confidence in the antibody.



What do the different pillars mean?

Genetic (knockout/knockdown)*

In these verification experiments, the target protein is knocked out or knocked down through genetic manipulation techniques, like CRISPR Cas9 or RNAi. The decrease or loss of antibody detection of the target protein relative to a control should correspond with the changes in expression for a verified antibody.

Overexpression*

Antibody specificity may be verified by overexpressing the target protein in an (ideally) exogenous system, or recombinantly expressing a tagged protein at endogenous levels. Antibody staining (e.g. an increase in measured signal) should correspond with different levels of expression for a verified antibody.

Orthogonal‡

In orthogonal verification, the staining results of antibody-centric experiments against a target are cross-referenced against the results from antibody-independent experiments (e.g. RNA-seq, 'omics techniques, *in situ* hybridization, etc.). Ideally, two samples with different expression levels are assayed and the orthogonal experiments correlate with one another for a verified antibody.

Independent antibody‡

Independent antibody verification experiments require that two independent antibodies with non-overlapping epitopes return matching staining patterns. The performance of the independent antibodies should compare favourably in two tissues or cell lines with different expression levels, and ultimately increase confidence in the specificity of the antibodies.

Capture mass spectrometry‡

Verification by capture mass spectrometry (e.g. immunoprecipitation mass spectrometry) relies on comparing the migration and immunostaining patterns of the target protein with the mass of the target when enriched from cell lysate by the antibody, as measured by mass spectrometry.

Cell treatment‡

In cell treatment verification experiments, cell treatment-induced changes to expression or target localization should be reflected in altered antibody staining patterns. Cell treatment might include the use of a drug or small molecule to effect those changes. Although cell treatment is not a pillar discussed in Uhlen M., *et al*[1], it is a technique adopted by some vendors to verify their antibodies.

Relative expression†

Relative expression verification experiments include comparing immunostaining patterns across different cell lines or tissues with the expected changes of basal expression levels of the target protein. Although relative expression is not a pillar discussed in Uhlen M., *et al*[1], it is a technique adopted by some vendors to verify their antibodies.

[1] Uhlen M, Bandrowski A, Carr S, et al. A proposal for validation of antibodies. Nat Methods. 2016;13(10):823-827. doi:10.1038/nmeth.3995

We appreciate the importance of evaluating and choosing verified antibodies, so as we continue to develop this feature on BenchSci, [we'd love to chat with you](#) about how you use verification data in your workflow.