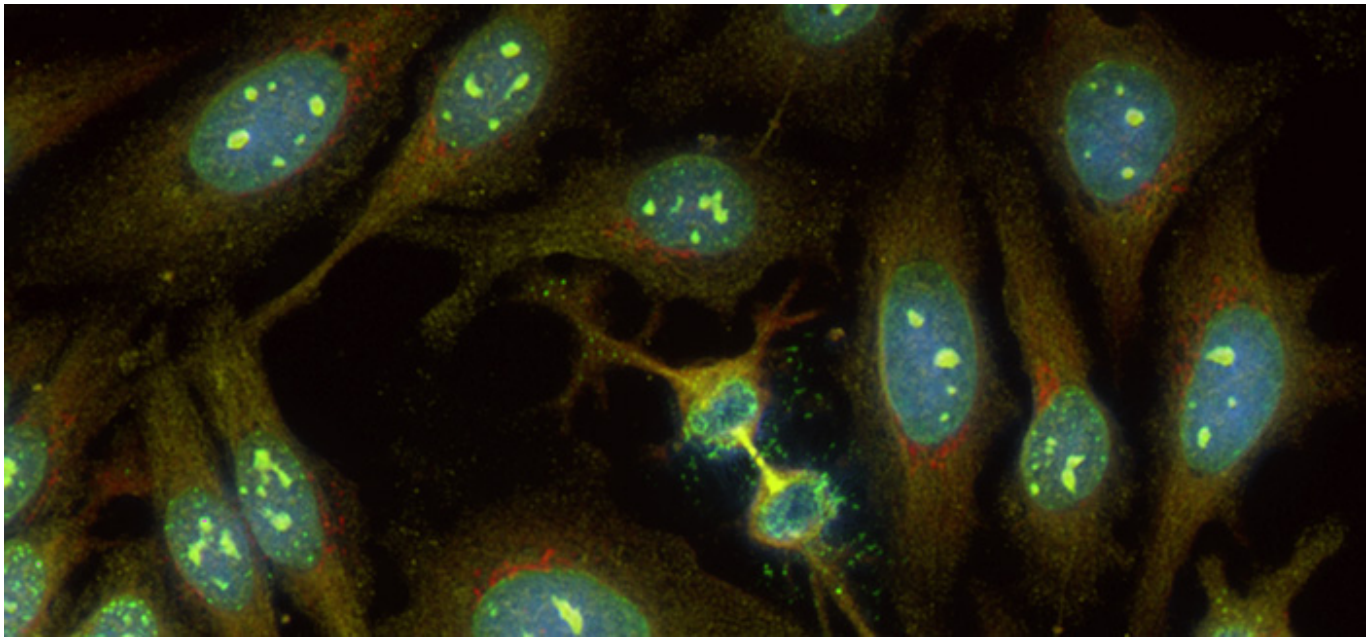
Secondary antibodies offer many advantages for scientific research. These include increased assay sensitivity, owing to the signal amplification from multiple secondaries binding to each primary antibody, and improved flexibility for experimental design, which results from the broad commercial availability of secondary reagents. However, not all secondary antibodies are created equal, and choosing the wrong product can lead to wasted time, money, and precious sample. To help you avoid common pitfalls, such as false-positive results due to non-specific binding, here are our top tips for secondary antibody selection.



Check the target species

The target species is, perhaps, the most obvious factor to consider when choosing a secondary antibody. For example, if your primary antibody was raised in a rabbit, you will need an anti-rabbit secondary antibody for its detection. But keep in mind that the secondary antibody should target the class or subclass of the primary antibody being used, especially when working with monoclonal primary antibodies, which belong to a single subclass. And remember that, if your primary antibody was raised in a hamster, you should determine the host strain (typically Syrian or Armenian), since cross-reaction between the different strains is not complete.

We offer secondary antibodies against a wide range of species – including alpaca, bovine, cat, chicken, dog, goat, guinea pig, hamster (Syrian and Armenian), horse, human, mouse, rabbit, rat, sheep, and swine – and our product portfolio covers an extensive selection of subclass-specific secondaries.

Select an appropriate host species

Selecting a suitable host species presents application-specific considerations. For multiple labeling, it is recommended to use secondary antibodies from the same host species to minimize interactions between different secondaries. If this is not possible, each secondary antibody should be cross-adsorbed against the host species of the other secondary antibodies being used (see Tip 5). For antibody capture assays, such as immunoprecipitation, knowing the affinity of Protein A and Protein G for different antibody species and subtypes will help guide product selection. Although personal preference may also influence your decision, we have found little difference in quality between secondary antibodies raised in different hosts.
Our extensive range of secondary antibodies includes products raised in alpaca, bovine, donkey, goat, mouse, rabbit, rat, and sheep.

Investigate the available conjugates

Secondary antibodies are usually supplied as conjugates – and the type of label that you choose will be driven by your application. Put simply, assays involving fluorescent detection require secondary antibodies labeled with fluorescent proteins or fluorescent dyes, while those based on chromogenic or chemiluminescent detection use enzyme-labeled secondary antibodies. Other available label types include biotin, colloidal gold, and Protein A/G. Alternatively, you may wish to purchase an unconjugated secondary antibody and label it yourself using an off-the-shelf conjugation kit. In this scenario, it is important that the antibody buffer is free of additives (e.g., BSA, azide, glycerol, Tris) that could interfere with the labeling reaction.

Our secondary antibodies are available unconjugated (in 0.01M sodium phosphate, 0.25M NaCl, pH 7.6), as well as labeled with horseradish peroxidase (HRP), alkaline phosphatase (AP), biotin, colloidal gold, and a choice of over 20 different fluorophores including Alexa Fluor® dyes.

Determine the purification method

Most secondary antibodies are purified from the serum of the host animal. This involves passing the serum through a column containing an immobilized capture ligand, commonly Protein A, Protein G or the target antigen (e.g., rabbit IgG), before eluting the bound antibody. A major advantage of using the target antigen for affinity purification is that only antigen-specific antibodies are captured, which reduces the risk of non-specific binding that could lead to unwanted background signal.

antibodies. *AffiniPure™ antibodies are affinity purified using target antigens coupled to agarose beads, ensuring high specificity, low background, and improved lot-to-lot consistency for more reproducible performance.*

Consider the need for cross-adsorption

Because antibodies from different host species often share similar structure and homology, a secondary antibody raised against the immunoglobulins of one species may also recognize those of another. This can result in non-specific signal. Cross-adsorption is similar to the affinity purification method described in Tip 4, but instead uses immobilized antibodies or serum proteins from potentially cross-reactive species. The use of cross-adsorbed secondary antibodies is recommended when performing multiple labeling experiments, staining antibody-rich cells or tissues, and running sandwich ELISAs based on indirect detection. However, caution should be exercised when considering antibodies that have been adsorbed against closely related species (e.g., mouse and rat) since they could have diminished epitope recognition. *Cross-adsorbed (min X) Secondary Antibodies have minimal cross-reactivity with species other than the intended target, making them ideal for multiple labeling applications, when staining antibody-rich cells or tissues, and when performing sandwich ELISAs based on indirect detection.*

Think about using a fragment antibody

Fragment antibodies are seeing increased use for scientific research, often to prevent non-specific binding to Fc receptors on immune cells. Popular formats include F(ab')2 fragments, which are generated by pepsin digestion of whole IgG antibodies to leave a bivalent molecule, and Fab fragments, which are produced using papain and have just a single antigen binding site. In addition, VHH fragments, consisting of the antigen-binding fragment of heavy chain-only antibodies, are produced in camelids and have a molecular weight of just 15 kDa, making them useful for cell and tissue imaging experiments, where good sample penetration is important.

We offer a comprehensive selection of fragment antibodies, including F(ab')2 Fragment Affinity Purified Antibodies, Fab Fragment Affinity Purified Antibodies, and AffiniPure-VHH™ Secondary Antibodies. Also, our FabuLight™ – Affinity-Purified Fc Specific Fab Fragments let you label primary antibodies prior to incubation with the sample, eliminating the need for a secondary antibody incubation step yet still providing signal amplification.

Choose a trusted supplier

Besides the antibody itself, you will want to evaluate its supplier. Factors to consider include product availability and expected lead times, which are critical for keeping your project on track, and the level of detail provided in datasheets and other resources, which can help to influence experimental design. Additionally, the quality of the technical support can be key to saving time, reagents, and sample material, as well as helping you to plan ahead.

on 40 years of experience in immunoglobulin purification, conjugation, and lyophilization. Contact us today to learn how we can support your research.

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