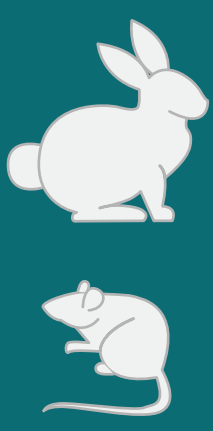
ROCKLAND

7 Considerations for Choosing the Best Secondary Antibody

1. Host Species
Once you have determined the host species used to generate the primary antibody, select a secondary antibody specific for detection of that primary antibody species. For example, when using a polyclonal antibody produced by a rabbit, you will select an anti-rabbit secondary antibody that was raised in an alternative host species such as mouse, goat, or donkey.



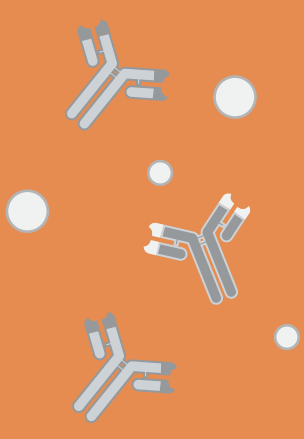
Most primary antibodies are produced in rabbit, mouse, goat, or chicken. Thus, anti-mouse, anti-rabbit, anti-goat, or anti-chicken polyclonal secondary antibodies are often used for detection. Remember, the species used to generate the secondary antibody should always be different from the host species of the primary antibody.

2. Reporters
After host selection, consider the intended detection assay. For commonly used techniques like Western blot, ELISA, or chromogenic Immunohistochemistry (IHC), an enzyme-conjugated secondary, such as peroxidase or alkaline phosphatase may be the best choice. For Immunofluorescence (IF) microscopy, Flow Cytometry, Fluorescence-Activated Cell Sorting (FACS), or if multiplexing and quantitation in Western blot is needed, then a secondary conjugated to a fluorochrome, such as FITC, DyLight™, or Cy™ dye should be used.

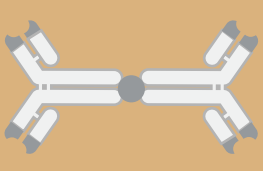
Biotinylated secondary antibodies can be used when amplification methods like the Avidin-Biotin Complex (ABC) or Linked Streptavidin-Biotin (LSAB) method are needed due to low expression of a target protein.

For immunoprecipitation experiments performed prior to Western blot (IP–Western blot), Rockland's TrueBlot® secondary antibodies are recommended. The unique detection properties of TrueBlot® can effectively eliminate interference from the heavy and light chains of the IP antibody.

3. Cross-adsorbed Antibodies
Pre-adsorption—also called cross-adsorption—is used to eliminate the immunoglobulin reactivity of undesired species, antibody fragments, and/or cell and tissue samples, which improves the specificity of the antibody.



The degree of cross reactivity is determined using ELISA or Western Blot detection and is typically less than 1% of the desired signal. The secondary antibody is cross-adsorbed against serum antibody protein from another species or is adsorbed against a mixture of serum antibody protein from several species (i.e., pre-adsorbed). These highly cross-adsorbed antibodies show low levels of cross reactivity necessary for multiple labeling experiments.



4. Class/Subclass
Primary polyclonal antibodies are generated in rabbit, goat, donkey, or chicken and are usually gamma chain immunoglobulins (IgG isotype). Therefore, the second ary antibody should be an anti-IgG antibody that recognizes both the heavy and light chain of the primary antibody (anti-IgG H&L).

On the other hand, primary monoclonal antibodies are normally raised in mouse, rat, and Armenian hamster but can also be rabbit or human-derived. Because monoclonal IgG antibodies are subclass specific, it's important to use a secondary antibody directed against that specific subclass.

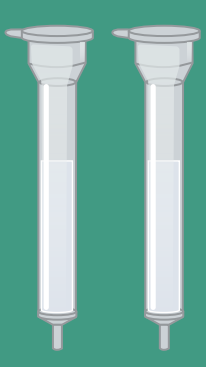
Despite the notion that any anti-mouse IgG should recognize any of the IgG subclasses, studies have shown potential bias toward specific subclasses. This makes the use of anti-mouse IgG subclass-specific essential to ensuring robust and consistent multiplex labeling of target proteins. However, when the sub-class of your primary antibody is unknown, use anti-IgG F(ab) or consider performing an isotyping assay.

5. F(ab')$_2$ and Fab Fragments
F(ab')$_2$ fragment secondary antibodies are generated by pepsin digestion of whole IgG antibodies. This process removes most of the Fc region while leaving the hinge region intact, resulting in a fragment divalent with MW ~110 kDa.

In contrast, Fab fragment secondary antibodies are generated by papain digestion of whole IgG antibodies. removing the Fc region entirely to generate a monovalent antibody of ~50 kDa.

Because of their small size, both F(ab')$_2$ and Fab fragment antibodies eliminate non-specific binding to the Fc receptors on cells and can penetrate tissue more efficiently. When working with tissues or cells that have Fc receptors like spleen, peripheral blood, hematopoietic cells, leukocytes, or NK cells, choose a F(ab')$_2$ or Fab to eliminate non-specific binding to Fc receptors.

These fragment-conjugated secondary antibodies are ideal for flow cytometry, immunohistochemistry, and immunofluorescence.



6. Affinity-purified Antibodies
Affinity-purified antibodies are isolated by separating monospecific antibodies from other antiserum proteins, while non-specific immunoglobulins are separated by solid phase affinity chromatography.

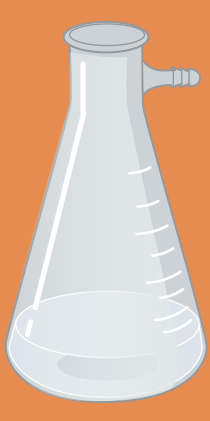
Using an affinity-purified antibody can increase specificity, improve sensitivity, lower background, and help maintain lot-to-lot consistency. This is because the affinity purification reduces variation from one product to another for more reproducible immunoassays.

Affinity-purified secondaries can also be used to optimize assays that show high background or non-specific binding from the secondary antibody.

7. IgG Fraction Antibodies
IgG fraction antibodies are robust and prepared by a combination of salt fractionation and chromatographic methods.

IgG fractions may provide antibodies with extremely high affinity, making for a more potent secondary antibody, whereas affinity-purified antibodies provide improved specificity but possibly at the expense of affinity.

Low abundance proteins or weakly detected primary antibodies that are detected using an affinity-purified secondary may be better recognized using an IgG fraction secondary.



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