Guide to Purification of Polyclonal Antibodies

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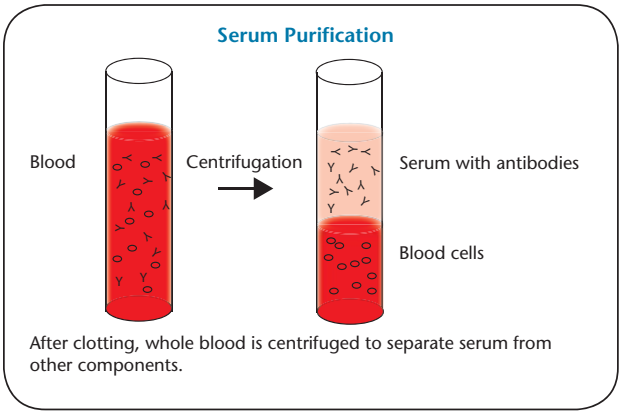
When choosing a polyclonal antibody, either as a primary or secondary antibody in an immunoassay, researchers are often inundated with an array of antibody purification terms that they may not know much about. Understanding polyclonal antibody purification steps gives researchers the opportunity to pick the most appropriate antibody purification technique for their particular immuno-application. Today, most polyclonal antibodies are referred to as being affinity purified. There are three major types of affinity purification: immunoglobulin-specific purification, antigen-specific purification, and serum adsorption. It should be noted that the different types of purification schemes are often used in combination to produce a better product, which is critical to the performance of the final immunoassay. But first, it is important to understand the early steps, serum purification and salt precipitation.

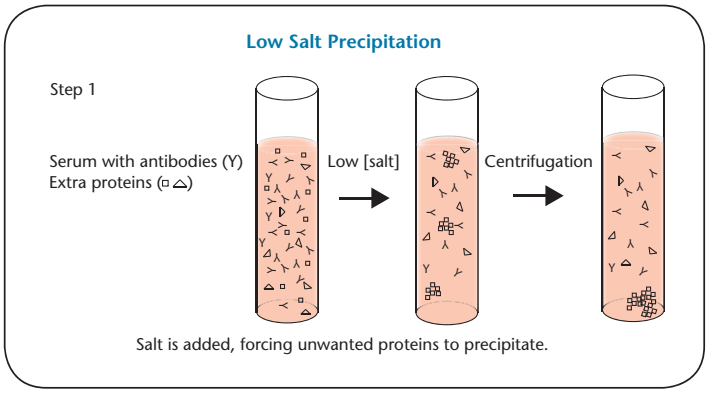
Serum Purification

Polyclonal secondary antibodies may be provided as a serum fraction. Serum is the amber colored supernatant obtained after blood is allowed to clot and is the simplest purification technique. Serum contains different types of antibodies as well as antibodies with a variety of affinities, some of which will bind to the antigen of interest and some of which will bind to non-specific antigens. Serum is inexpensive and provides sensitivity, but not reliable specificity. In fact, serum will likely contain less than 10% specific antibody. The lack of specificity is due to a multitude of antibody clones and other proteins, including albumin, which may bind non-specifically in an immunoassay. Serum will often be found in individual labs provided by researchers developing their own antibodies.

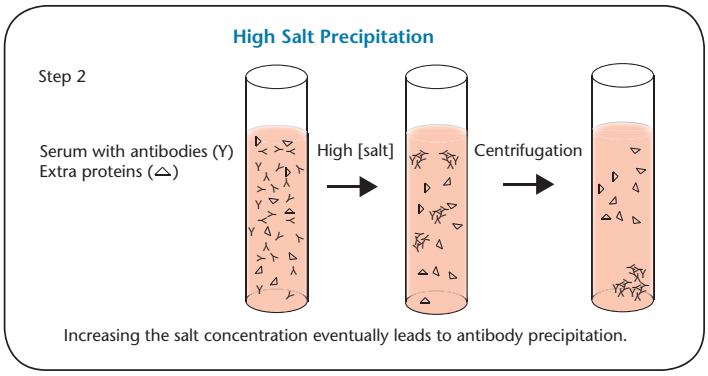


Salt Precipitation

One common technique for purifying proteins is salt precipitation. The solubility of proteins is related to the salt concentration of the solution. Increasing the amount of salt in a solution essentially removes water molecules from the protein, causing the protein to precipitate. This is commonly called “salting out.” The



differences in composition and shape of proteins mean that proteins precipitate at varying concentrations of salt. Often proteins are precipitated in a two-step or fractionated process. In the first step, salt is added so that the antibody of interest remains soluble, and unwanted proteins are removed. In the second step, more salt is added so that the antibody of interest precipitates, and the unwanted soluble proteins can be removed. The precipitate is then dialyzed to remove the salt, and the antibody dissolved back into solution. Serum can be precipitated this way using ammonium sulfate or caprylic acid. Salt precipitation is an inexpensive way to purify antibody from serum; however, the process does not remove proteins with a precipitation pattern similar to antibodies.

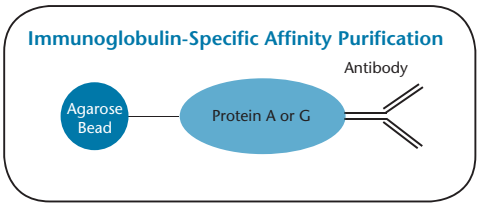


Affinity Purification

There are three types of affinity purification. Many times, the three are used in combination when the purest antibody is needed.

Immunoglobulin-Specific Affinity Purification

In certain bacteria, immunoglobulin-binding proteins are used to evade the host immune response. These proteins bind immunoglobulins in an orientation that prevents normal antibody function. Repurposed for purification, immunoglobulin-binding proteins are used to increase the purity or to concentrate an antibody solution.



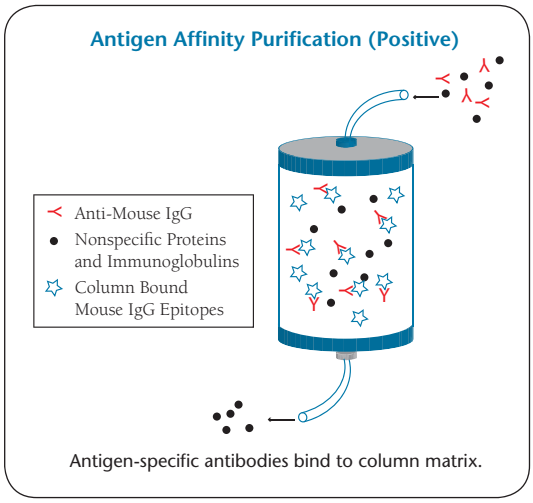
There are three common immunoglobulin-binding proteins used to purify antibodies, Protein A, Protein G, and Protein L. Protein A (from *Staphylococcus aureus*) and Protein G (from *Streptococcus* spp.) bind to the Fc region of antibodies while Protein L (from *Peptostreptococcus magnus*) binds to the antibodies through the κ light chain. Often these proteins are immobilized to a solid support (e.g., agarose beads) to form affinity matrices. Protein A, Protein G, and Protein L all have unique immunoglobulin-binding characteristics according to species, antibody type, and antibody isotype. Care must be taken when selecting an immunoglobulin-specific purification to make sure the antibody of interest will actually bind to the immunoglobulin-binding protein. Because the technique does not isolate antigen-specific antibodies, immunoassays using immunoglobulin-purified antibodies may still have background or specificity issues.

It should be noted that immunoglobulin-specific purification is a specific type of affinity purification; however, manufacturers often do not distinguish between Protein A, Protein G, or Protein L purification versus antigen affinity purification.

Antigen Affinity Purification

Antigen affinity purification is another specific type of affinity purification and results in the purest antibodies with the least amount of cross-reactivity. Affinity purified antibodies exhibit the highest specificity and sensitivity that can be obtained from serum. Because these antigen-specific antibodies are polyclonal, they can be used as both the capture and detection antibody in capture (sandwich) immunoassays.

Despite all the care used to purify antibodies against a specific antigen there are some epitopes that are shared between proteins. These shared epitopes can cause cross-reactivity. In order to remove the cross-reactivity, negative affinity selection is utilized. Negative affinity purification selection is similar to antigen affinity purification except that unwanted proteins are used to remove cross-reacting antibodies. Often the unwanted proteins are attached to a solid support, and an affinity column is constructed using the cross-reacting antigen. During the purification process a negative affinity column is utilized to remove the unwanted antibody. Negative selection can be performed one or more times depending on the level of purity needed. The caveat to negative affinity columns is that the total number of epitopes being recognized by the polyclonal antibody is being reduced, and thus, the sensitivity will begin to decrease. A tradeoff must be made between sensitivity and specificity.

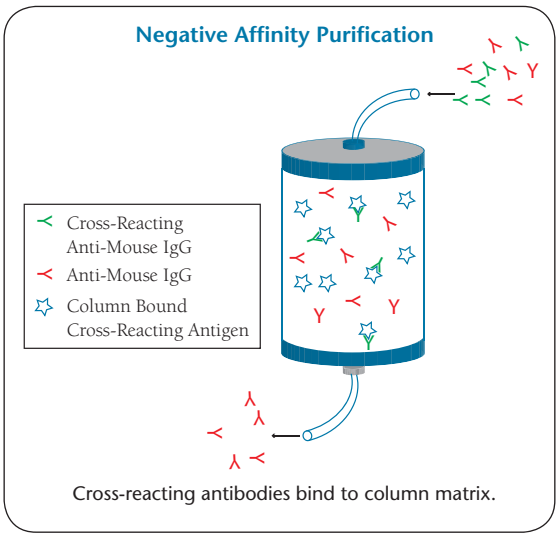


Serum Adsorption (Negative Affinity Purification)

Polyclonal antibodies are most commonly used as anti-immunoglobulin secondary antibodies in various immunoassays. Even though polyclonal antibodies often display higher sensitivity than monoclonal antibodies, they can suffer from a lack of specificity. In certain applications even antigen affinity purified antibodies may suffer from a lack of specificity. Despite being affinity purified as species-specific, anti-immunoglobulins can bind other species to varying degrees due to shared epitopes across species. Depending on the immunoassays, researchers may need this cross-reactivity removed. Traditionally, the cross-reactivity was removed by adding serum of the unwanted species. The cross-reacting antibodies bound to the serum and were removed from the total antibody pool. Cross adsorption is easy to perform but introduces a complex protein mixture of serum from a different species back into a relative pure antibody solution. The antibody must then be repurified.

While the above technique is historically valid, these days most manufactures of secondary antibodies attach serum to a solid support and make negative affinity columns from serum. Thus serum adsorption is better categorized as a specific type of negative affinity, rather than a purification technique.

Despite the wide use of negative affinity for purification purposes, manufacturers have traditionally only listed which antibodies have been serum adsorbed because knowledge of serum adsorption is critical for researchers performing multiplex assays.



Cross-reacting antibodies bind to column matrix.

How KPL Purifies its Antibodies

KPL pioneered the development of large-scale affinity purification and was the first company to commercialize affinity purified antibodies. KPL's proprietary purification technology results in higher affinity antibodies, which increases sensitivity and reduces background. Selected antibodies may be further purified using negative affinity columns to minimize cross-reactivity between animal species or to reduce shared reactivity with other immunoglobulin classes. These processes yield pure antibodies with defined specificities and results in consistent lot-to-lot performance.

KPL's affinity purified antibodies are available either unlabeled or labeled with enzymes, tags, and dyes [including alkaline phosphatase (AP), horseradish peroxidase (HRP), biotin, fluorescein (FITC), rhodamine (TRITC), Cy™ Dyes, DyLight™ dyes, R-phycoerythrin (R-PE), colloidal gold, latex beads, or magnetic beads]. They are ideal for use in immunoassay applications such as ELISA, Western blotting, and immunohistochemistry.

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