Purification and Modification of Antibodies

Purification of antibodies from either supernatant or serum involves the separation of the antibodies from other proteins. Whilst some assays do not require the use of purified antibodies, purification is often required to avoid unwanted interactions with other protein molecules and components.

Antibody purification is a selective process and the following methods can be used to produce purified antibody. All methods are available to be included as part of a Bulk Antibody or Custom Polyclonal or Monoclonal Antibody Production Project.

Protein Purification

Purification of IgG is typically carried out by Protein A or Protein G affinity purification.

Protein A, G and L are useful tools for purification of the total IgG fraction:

Protein A	is a component of *Staphylococcus aureus* located in the cell wall. Protein A binds to the Fc region of immunoglobulins, specifically IgG. This ability varies between species and isotypes. Protein A is a very efficient and cost-effective method of purification and is the method of choice for rabbit polyclonal projects to purify total IgG.
Protein G	is a protein isolated from group B streptococci, again found in the bacterial cell wall. Protein G binds to most mammalian immunoglobulins through the Fc regions but bind strongly to all human and mouse subclasses. This makes Protein G suitable for purification where Protein A purification is not effective at neutral pH.
Protein L	is a surface component from *Peptostreptococcus magnus* but, unlike Protein A and G, it does not bind through the Fc region but through light chains, specifically subtypes of kappa light chains. The usefulness of Protein L for purification purposes is limited to antibodies where the light chain isotope has already been determined.

Protein A and Protein G are both microbial proteins expressed on the cell surface of Staphylococcal (Protein A) and Streptococcal (Protein G) bacteria. These proteins function to bind host immunoglobulin at the Fc region. These binding properties also make Protein A and Protein G ideal for antibody purification using chromatography systems.

Pre-clarified supernatant or antisera are introduced into columns containing protein A or G, immobilized on agarose beads. The antibody will bind to the Protein A or G. The resultant purified antibody may then be eluted from the column after a washing step.

Once purified the antibody is buffer exchanged and gently concentrated to the required concentration as necessary.

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Affinity purification

A further option for purification is to use the antigen to bind immunoglobulins since all other components of the solution may be discarded. Using this method, non-specific immunoglobulins are also discarded.

Ammonium Sulphate Precipitation

Ammonium sulphate precipitation is frequently adopted as a method to concentrate antibodies from serum or cell culture supernatant since antibodies precipitate at lower concentrations of ammonium sulphate than other proteins and components of serum. This is often used as a primary step prior to other forms of purification.

Dialysis and Ultrafiltration

This method may be adopted to separate immunoglobulins from other proteins due to the molecular weight of immunoglobulins. However, this process will not remove proteins of a similar molecular weight to immunoglobulins (c. 140 kDa) and is therefore frequently used as a buffer exchange/concentration step together with another process.

IgM Purification

Class M immunoglobulins (IgM) bind very weakly to Protein A or Protein G. However, Protein L may be used but this will also bind IgG showing the same light chain subtype. IgM is typically purified using a combination of purification techniques, such as ammonium sulphate precipitation followed by IEC.

IgY Purification

IgY are a class of immunoglobulins, similar in action to IgG, but particular to birds, reptiles and some fish. Standard Protein A and Protein G purification techniques are not effective to purify IgY. IgY is usually purified from the yolk of eggs produced by the host animal and a selection of purification methods may be adopted, including precipitation techniques followed by IEC.

Further processing and modification

Endotoxin testing	Endotoxin testing may be performed using LAL (Limulus Amoebocyte Lysate) assays.
Labelling	Purified antibodies may be labelled with a selection of labels, including horseradish peroxidase (HRP) and alkaline phosphatase (AP), fluorochromes such as fluorescein and fluorescein isothiocyanate (FITC) and biotin. Labelling is carried out using commercially available kits ad a full listing if the available labels is available from our Technical team.

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Modification	Antibodies may be modified by fragmentation with the enzymes papain to yield the Fab fragment, or pepsin to yield the F(ab')2 fragment.

Our antibodies can be purified, modified and presented in a form to suit you. Please contact our Technical Team for more information.

Support

Antibody Production Services is a division of Life Science Group Ltd.

To learn more, contact us:

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