ANTIBODY **PURIFICATION**

ANTIGEN AFFINITY PURIFICATION
CLASS - SPECIFIC AFFINITY PURIFICATION
PHYSICO-CHEMICAL SEPARATION

Expert in Antibody Engineering

From Research to Discovery

ISO 9001 : 2015 certified

covalab
R&D in Biotechnology

CLASS-SPECIFIC AFFINITY PURIFICATION

Principle

Upon evolution, some microorganisms have developed the ability to produce immunoglobulin-binding proteins which helps them circumvent the immune system of their host. Such proteins bind immunoglobulins through their Fc region and thus prevent their recognition by the antigen-binding domain.

We take advantage of this property by using those proteins in adapted devices to bind immunoglobulins selectively.

Protein A : *The "Golden" standard purification*
Protein A binds to the Fc region of immunoglobulins, especially IgG. The interaction with IgG vary between species, and even within a species. Thanks to its low price, this protein is widely used for large-scale purification of cell culture supernatants and ascitic fluids, and usually yields high purity and recovery rate.

Protein G : *The protein A alternative*
Protein G is a bacterial cell wall protein isolated from group B streptococci. This protein binds to most mammalian immunoglobulins, and more specifically to all human and mouse subclasses, through their Fc regions.

Protein L : *The answer to rat's immunoglobulin purification*
Protein L is able to bind not only a wider range of antibody classes including IgA, IgD, IgE, IgG and IgM but also antigen-binding antibody fragments such as Fab, F(ab')2 or scFV.

Advantages

Nowadays, engineered as well as hybrid forms of these proteins are used as immunological tools.

In addition to their ease of use and cost effectiveness, their ability to bind antibodies regardless of their antigenic specificity has made them a very powerful and flexible tool for antibody purification.

Why perform class-specific affinity purification with us ?

Depending on their host species, class and sublass, those proteins bind antibodies with variable affinity, and sometimes not at all.

Our experience in antibody engineering and protein purification allows us to select the best protein to use and optimise the purification conditions within a short period of time.

Expert in purification on protein A? Do it yourself with our kits!

Product	Packaging	Sample Volume	Max number of runs/column	ID Covalab	Ref.
Maxi protein A Purification kit	2 columns	According to your needs	9	kit0003	00116577
µ-spin affinity protein A purification Midi kit	5 columns	750 µl	3	kit0005	00117239
	20 columns	750 µl	3	kit0005	00117241
µ-spin affinity protein A purification Mini kit	5 columns	200 µl	3	kit0006	00117585
	20 columns	200 µl	3	kit0006	00117587

For more details, contact us or read our "Antibody purification kits" flyer!

ANTIBODY PURIFICATION

Our engineers offer high quality purification, whether you have a project in progress or you simply need our expertise.

Antibody purification involves selective separation of antibodies from other plasma components (polyclonal antibodies), or from ascitic fluid or cell culture supernatant after large scale production using hybridomas (monoclonal antibodies).

We carry out various methods to purify antibodies ranging from poorly to highly specific ones.

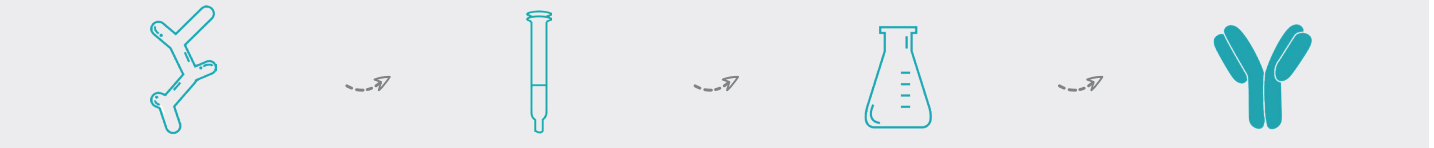
COVALAB, YOUR PARTNER IN ANTIBODY ENGINEERING SINCE 1995

MADE IN FRANCE - ISO 9001 : 2015 CERTIFIED

ANTIGEN AFFINITY PURIFICATION

Principle

By immobilizing the antigen of interest (e.g. the peptide used to raise the antibody) on a solid phase, the antibodies that bind specifically to the antigen are retained upon loading of the serum, while other impurities as well as unspecific immunoglobulins are discarded in the flow-through.



Step 1
The antigen is immobilized on agarose beads with precise orientation and put into the column.

Step 2
The sample is loaded into the column. Specific antibodies bind to the resin or agarose beads while others and impurities are discarded.

Step 3
The column is eluted using acidic buffer. Specific antibodies are released from the agarose beads.

Step 4
Specific antibodies are checked by ELISA against the antigen to confirm their specificity.

Advantages

Immunoaffinity provides many advantages such as very high purity levels (>99% can be achieved in one step), very high selectivity and therefore very high resolution including certitude that the antibody is specific for the antigen of interest.

Why perform antigen affinity purification with us ?

Antigen orientation may be a concern especially in the case of peptides, which could be not easily recognised by antibodies if major binding sites are sterically hindered by the chemical bonds with the agarose beads.

Thanks to our expertise in antibody and peptide chemistry, we are able to determine the best conditions to allow the most effective antigen-antibody interaction and ensure the best purification yield.

Anti-PTM (Post-Translational Modification) antibodies purification

Antibodies raised against a modified peptide need to undergo an additional step to discard immunoglobulins that are specific for the unmodified counterpart prior to purification.

Step 1 : The immune serum is loaded onto a column with the **control peptide** coupled to agarose beads.
Step 2: The flow-through is loaded onto a column with the **modified peptide** coupled to agarose beads.
Step 3: The eluate is checked by ELISA against **both peptides** to control its immunoreactivity.

PHYSICO-CHEMICAL SEPARATION

Principle

This method involves combinations of unspecific techniques based on the physicochemical properties of immunoglobulins to isolate them from other components of serum or cell culture medium.

Such properties are hydrophobicity, apparent size or isoelectric point.

Ammonium sulphate precipitation

Because of their higher hydrophobicity, antibodies precipitate at lower concentrations of ammonium sulphate than most proteins and other serum components. Ammonium sulphate precipitation is then frequently used to enrich and concentrate antibodies from serum, ascitic fluid or cell culture supernatant.

Size exclusion

Dialysis membranes, size exclusion resins, and diafiltration devices that feature average molecular weight cut-offs can be used to separate immunoglobulins (>140kDa) from small proteins and peptides. Gel filtration and dialysis are more commonly used following other purification steps, such as ammonium sulphate precipitation.

Ion exchange chromatography

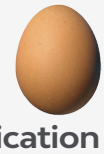
Ion exchange chromatography (IEC) uses positively or negatively charged resins to bind proteins based on their net surface charge. This method allows to purify antibodies and proteins with high resolution. Once optimized, IEC is a cost-effective, gentle and reliable method of antibody purification.

Purification of IgM

IgM naturally form pentamers bound together through the Fc region. Therefore protein A and protein G are almost unable to bind them.

Protein L could be of great use in this case, but other classes of immunoglobulins that have the same isotype of light chain would be bound as well.

IgM are then usually purified by ammonium sulphate precipitation followed either by ion exchange chromatography or by IgG depletion using protein A or G affinity.



Purification of IgY

IgY are the major class of immunoglobulins of birds and reptiles. They have the same function as IgG, but differ significantly in the Fc region, preventing protein A and protein G from binding.

Protein L is also useless since chicken do not have kappa light chains.

Selective precipitation is then the most used technique to purify IgY, followed if necessary by ion exchange chromatography when used in sensitive applications.

Advantages

Although physicochemistry-based purification techniques are poorly selective when compared to class-specific affinity or antigen affinity. These techniques are easy to set up and perform in routine, and are also more cost-effective!

Why perform physico-chemical separation with us ?

While the other two methods based on protein-protein interaction, only chemistry is the key to perform efficient and reliable physico-chemical separation.

Our experienced chemists are able to set the best parameters within the shortest time. Even hard-to-purify antibodies such as chicken IgY can be isolted using our well-proven procedures.

CUSTOM PURIFICATION

Till labelling or just HPSEC, make up your own purification program to meet your specific needs. Because each customer and project is different, we assist you step by step and offer complete or partial support adapted to your needs.

1 PURIFICATION

ANTIGEN AFFINITY
OR
CLASS-SPECIFIC AFFINITY

STOP

2 POLISHING

SIZE EXCLUSION CHROMATOGRAPHY
ION EXCHANGE CHROMATOGRAPHY

STOP

3 CARACTERIZATION

QUALITY CONTROL
Validation by SDS-Page / Western Blot
Validation by flow cytometry
Validation by HPSEC (High Pressure Size Exclusion Chromatography)
Validation by ELISA

SPECTROPHOTOMETRIC QUANTIFICATION
Determination by Do280
Determination by BCA

STOP

4 ADDITIONAL SERVICES

LABELLING
BUFFER EXCHANGE
ENDOTOXINS TEST
...
MORE UPON REQUEST

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