Mass Spectrometry & Purification Techniques

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Perspective

Antibody Purification Methods Based On Classification

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DESCRIPTION

In the fields of biochemistry, cell biology, and immunology, antibodies have emerged as a common and essential tool. Antibodies and antibody fragments come in a wide variety of forms and are employed in numerous applications. To produce antibodies with the required specificity and sensitivity, numerous alternative purification procedures have been created. Polyclonal antibodies, ascites fluid, or cell culture supernatant of a hybridoma cell line are sources of antibodies that are purified by selective enrichment or specific isolation (monoclonal antibodies).

METHODS FOR ANTIBODY PURIFICATION

Physicochemical fractionation

Immunoglobulins may differentially precipitate, size-exclude, or attach to solid surfaces based on the size, charge, or other chemical properties that antibodies share in typical samples. The immunoglobulins are among the sample proteins that are isolated.

Class-specific affinity

Certain biological ligands that are immobilised and have an affinity for immunoglobulins, such as proteins or lectins, bind to specific antibody classes in solid phase, such as IgG. Without regard to antigen specificity, this cleanses all antibodies belonging to the target class.

Antigen-specific affinity

Only antibodies in a sample that attach to a specific antigen molecule *via* their unique antigen-binding domains are purified using affinity. Without regard to antibody class or isotype, this cleanses all antibodies that bind the antigen.

Since the target antibody is (for all intents and purposes) the only immunoglobulin present in the production sample, antibodies that were created as monoclonal antibody hybridoma cell lines and produced as ascites fluid or cell culture supernatant can be entirely purified without utilising an antigen-specific affinity approach (third type). Contrarily, antigen-specific affinity purification is necessary for polyclonal antibodies (serum samples) in order to prevent the co-purification of non-specific immunoglobulins. The percentage of total IgG that is typically specific for the antigen used to immunise the mouse is typically between 3 and 6 percent. The kind and levels of purification required to produce a viable antibody depend on the applications for which the antibody is intended.

Purification based on their classification

Structures in antibodies have been preserved *via* evolution. In order to isolate antibodies based on the commonalities among each class, many techniques have been devised.

Protein A, G, and L: Bacterial proteins A, G, and L have particular domains that bind to particular immunoglobulins. Proteins A and G bind to the Fc region, while protein L binds to the light chain of the Fab region. Each protein has unique binding characteristics.

Purification of IgM: IgM's binding sites are sterically hindered, therefore proteins G and A do not bind to it robustly. Ion exchange chromatography, gel filtration, zone electrophoresis, and ammonium sulphate precipitation are some of the techniques used to purify IgM.

Purification of IgA: When a D-galactose lectin named jacalin was taken out of jackfruit seeds, this technique was discovered. It has been discovered that this substance binds to IgA and has four identical domains. IgA can be purified and separated from other immunoglobulins using jacalin, which can be immobilised on agarose gels.

Purification of IgY: IgY, a special immunoglobulin produced only by hens, is widely distributed in egg yolks. Since proteins A, G, and L do not bind to IgY, they cannot be utilised to purify IgY. So, IgY is purified using the ammonium sulphate precipitation method.

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CONCLUSION

Antibodies can be extracted from serum and cell culture supernatants using the antibody purification method. If an antibody fragment has a portion that interacts with the ligand bound to the matrix, it can be isolated. Affinity chromatography can be used to purify scFv, Fab, and dAb equally well. For applications that don't require the utmost purity, antibody purification can be the only purification step. Depending on the purpose, further purification procedures may be required.