

abcam

Antibody production

Laboratory production of antibodies for research use.

Overview

The production of antibodies relies on the in vivo humoral response to injected foreign antigens. Simple immunizations of many foreign molecules, viruses or cells can elicit a strong antibody response, but some substances fail to induce a strong response. The immune system can be manipulated to increase the response by modifying either the antigen or the host. Until recently these modifications were empirically discovered, but increased understanding of the immune system now allows for a more rational approach.

Proteins, peptides, carbohydrates, nucleic acids, lipids and many other naturally occurring or synthetic compounds act as successful immunogens. Peptides and non-protein antigens usually need to be conjugated to a carrier protein (bovine serum albumin or keyhole limpet hemocyanin) to become good immunogens. This is due to their small size, and conjugation to the carrier provides the required class II T receptor binding sites. Additionally, immunogens may need to be administered with an adjuvant to ensure a high quality/quantity response. Adjuvants are non-specific stimulators of the immune response. They allow smaller doses of antigen to be used to elicit a persistent antibody response.

Polyclonal production

Polyclonal antibodies are made by immunizing with an antigen. Repeated immunizations of the same antigen at intervals of several weeks stimulates specific B cells to produce large amounts of the anti-antigen. The blood will contain a variety of antibodies, each to a different epitope on the antigen. The immune-sera can be used in its crude form, where high levels of specific antibodies are present, or the specific antibodies can be isolated from sera components by affinity purification.

Monoclonal production

To produce monoclonals the same immunization protocol is used and all antibody-forming cells (e.g. B cells) are removed. These are fused with immortal tumor cells to become hybridomas, which are screened for antibody production and performance. The hybridomas that produce antibodies are given clone names, which are uniquely assigned to permit identification. The antibody producing hybridoma cells are cloned by isolation and cultivated using tissue culture. Alternatively, genes coding for antibody production can be cloned into transfection vectors to produce recombinant antibodies. Unlike polyclonal antibodies, monoclonals are homogenous with defined specificity to one epitope. The antibody secreted by the cells into the culture media can be harvested and used in its crude form, or it can be purified by affinity chromatography.