

Antigen Selection for Custom Antibody Production

An immunogen is an antigen or any substance that may be specifically bound by components of the immune system. The immunogenicity of the chosen antigen is of paramount importance to the success of any custom antibody project. The characteristics of the antigen must be determined as this will impact the choice of host and protocol used to create the antibody and the eventual success of the project.

Whatever antigen is used there will probably be multiple epitopes against which antibodies could be generated. When choosing epitopes for your antibody production it is best to look for those with exposed hydrophilic regions as these will be easily accessible for antibody binding. Hydrophobic regions are usually hidden within the protein.

Sources of Antigen

Antigens can be derived from a variety of sources:

1. Proteins:

Protein antigens can be either naturally occurring or recombinant. When choosing your protein antigen you should consider the following:-

- The size of the protein, the amount of aggregation and the relative nativity of the protein can all affect the quality and quantity of antibody produced.
- Generally, the larger the immunogenic protein the better.
- Soluble, non-aggregated proteins may induce tolerance in the host rather than a satisfactory antibody response.
- Antibodies to native proteins react best with native proteins and antibodies to denatured proteins react best with denatured proteins. For example if antibodies are to be used on Western/immuno blots, or other assays where the proteins are subjected to denaturing conditions, then antibodies should be made against denatured proteins.
- If antibodies are to be used to react with a native protein or to block a protein's active site, then antibodies should be made against the native protein.

2. Bacteria/Viruses:

Live bacteria or viruses, should not be used except as a last resort. There are many fixatives and inactivation techniques and one can generally be found that does not damage the antigenic determinants.

3. Plant and Yeast Antigens:

Sometimes high backgrounds can be found in host animal sera for plant or yeast antigens. We can supply pre-immune serum for screening for selecting candidate animals for immunisation.

4. Other Antigens:

Small polypeptides, polysaccharides and other non-protein antigens generally need to be conjugated or cross-linked to larger, immunogenic carrier protein such as KLH to increase immunogenicity.

Antigen Preparation

With the selection of a suitable antigen being critical to the successful initiation of an immune response in the host animal, we can provide immunogen preparation services, including peptide synthesis and carrier protein conjugation.

- Antigens should always be prepared using techniques that ensure that they are free of microbial contamination. Most protein antigen preparations can be sterilized using a 0.22 micron filter. Endotoxin levels should be determined.
- Where possible, antigens should be free of preparative by-products and chemicals such as polyacrylamide gel, urea, endotoxin, particulate matter and extremes of pH.
- The buffer used to re-suspend your protein is important as some buffers may have an adverse effects on the animal's welfare. The least toxic option available should be used. PBS is recommended.
- The final immunogen used is an oil/water emulsion so therefore detergents should be avoided if possible as these may affect the stability. SDS can be used provided the concentration is less than 0.1%.
- APS can accept antigens as either lyophilised or liquid material. Antigens may be also be provided prepared as a conjugate to a carrier protein such as KLH. This is also a service option available as part of a custom project.
- Carrier Protein Conjugation:
A standard peptide of 15-20 amino acids in length is too small to elicit an immune response on its own. As a result, it is necessary to conjugate the peptide to a larger carrier protein such as KLH. We can accept antigens as lyophilised or liquid material, which can be conjugated to a carrier protein or we can provide this service for you.
- Adjuvant:
The adjuvant, Complete Freund's, is typically used for Primary immunisation, with subsequent immunisations using Incomplete Freund's. In order to eliminate or reduce discomfort, APS pre-screens batches of adjuvant and reserves suitable batches. A number of other adjuvants are available on request.

Support

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

To learn more, contact us:

Telephone: +44 (0) 1234 889180

Email: sales@lifesciencegroup.co.uk

Website: www.antibodyproduction.co.uk

Address: PO Box 1519, Bedford, United Kingdom