

Important Immunogen Factors To Consider

Proteins, peptides, carbohydrates, nucleic acids, enzymes and lipids can all act as successful immunogens. The following characteristics help determine the immunogenicity of your molecule:

- **Size of the molecule and/or sequence information**

Molecules less than 3-8 kD are generally not good immunogens without further modification. Conjugation to a carrier protein such as KLH or BSA will increase the immunogenicity of the antigen. MBS provides conjugation services on site.

- **Immunogenicity**

To generate a MAb to a particular molecule, the epitope must be recognized by the host.

- **Stability**

Immunogens must be stable and able to withstand specified storage conditions for 6-12 months.

- **Quantity**

For a normal mouse hybridoma project, the amount of immunogen needed for start-up is ~3.0mg of immunogen and 1-2mg of screening antigen. This will complete the immunization stage, including screenings of serum and post-fusion supernatants. It is important to start with an adequate amount, as it reduces the lot-to-lot variability caused by new antigen preparation.

- **Quality**

In the preparation of an immunogen, it is essential that it be of high quality to reduce the generation of irrelevant antibodies. If a mixed population of antigens is used for the immunizations, then an antibody response to several components of the preparation is possible. If all of the compounds in a preparation are equally immunogenic, the resulting antibodies will mimic this distribution.

- **Homology**

The origin of the molecule is very important because this will help us to determine the best host animal for immunizations (i.e. rat or mouse). For example, there is often >95% homology between mouse and human proteins. Increasing the number of recognizable epitopes increases the chances of generating a useable monoclonal antibody.

- **Controls**

A positive control specific to the target analyte is important, as it helps optimize the screening process. This can be a commercially available polyclonal or monoclonal antibody.

Depending on your project goals, you may want to discuss an alternate immunization protocol or the use of a different adjuvant that may reduce the amount of immunogen required. Our standard protocol is designed to deliver the highest probability of success for a typical set of starting conditions. However, we are willing to discuss tailoring the project to your needs.

At the conclusion of the initiation phase of your project, all paperwork is reviewed, a master file is started, animals are set aside and antigen is aliquoted. A pre-immunization bleed is taken to use for a negative control.