Generating ultra-specific custom monoclonal antibodies in just eight weeks

Human Combinatorial Antibody Library (HuCAL) technology is not only fast, it also allows direct development of ultraspecific antibodies through guided selection protocols. This includes monoclonal antibodies that can recognize a single amino acid difference, a single amino acid modification, or either of two 10-amino-acid cleavage products but not the 20-amino-acid full-length peptide.

AbD Serotec provides monoclonal antibody development using the HuCAL® Gold recombinant antibody library, which offers many unique advantages over the use of animals for monoclonal antibody generation. In addition to being fast—the library’s more than 15 billion antibody specificities can be screened in less than six weeks—this phage display–based technology allows the selection process to be modified to direct the generation of exquisitely specific antibodies.

Guiding the selection procedure
Generating a highly specific antibody using animals can be a long process. As antibody generation inside the animal cannot be controlled, most antibody projects require the use of multiple animals to generate as many different antibodies as possible, which must then be laboriously screened to determine their specificity.

HuCAL is both naive and synthetic and was developed by MorphoSys for the generation of therapeutic antibodies against drug targets. The library was designed in silico based on sequence information obtained from bioinformatic analysis of the human immune system, and is based on a modular system of 49 framework genes and 6 highly variable complementarity-determining region fragments. With HuCAL, more than 15 billion high-quality antibody specificities are ready and waiting to be screened against antigens in vitro. This in vitro antibody selection process is the key to the power of this technology, as it can be guided to find the exact specificity needed. Intelligent blocking and subtraction strategies can be used to select epitope-specific antibodies that can either distinguish between closely related antigens or recognize multiple antigens. Alternately, antigens may be denatured, captured or masked to ensure that the selected antibodies recognize the antigen only under specific assay conditions.

Example 1: modified amino acids
Initially, the HuCAL library is incubated with the unmodified form of a peptide to remove the antibodies that recognize it. At this time, other related peptides are added (Fig. 1) to improve the final specificity. This depleted library is then screened with a peptide displaying the desired modification (for example, phosphorylated or oxidized amino acids).

![Figure 1](image-url)

Figure 1 | Direct selection of a sequence- and phosphorylation-specific antibody. The HuCAL library was preadsorbed with related peptides that were either nonphosphorylated or phosphorylated at the same amino acid but with an alternate flanking sequence (top). The depleted library was then screened against the desired phosphorylated peptide (peptide A). The target peptide was conjugated to both transferrin and BSA carriers for screening, to eliminate carrier-specific antibodies. Three antibodies recognizing only the phosphorylated target peptide were isolated and checked by enzyme-linked immunosorbent assay (ELISA; bottom).

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to generate specific antibodies. Once the antibodies have been tested for specificity by ELISA against the target and both related and unrelated peptides, they are produced in larger scale ready for use. The entire process takes just eight weeks.

Example 2: highly conserved antigens
The blocking and subtraction strategy described above is also ideal for selecting specific antibodies when the antigen is highly conserved, such as a protein that varies only slightly between species or has a single amino acid mutation (Fig. 2). If both the target and the related protein are available, the library can be simply depleted of unwanted specificities. If one or more of the proteins are unavailable, AbD Serotec assists with the identification of suitable regions for fragment expression (see below) or peptide synthesis.

Example 3: distinguishing parts from the whole
The generation of antibodies able to recognize a cleavage product but not the full-length antigen also demonstrates the power of this technology. In this case, screening the HuCAL library generated 4 antibodies, each recognizing one of the two 10-amino-acid cleavage peptides (2 antibodies per peptide), without recognizing the full length 20-amino-acid peptide. As in the cases above, the undesired specificity, in this case the full-length peptide, was used to deplete the library of the unwanted antibody specificities before screening with each of the target peptides.

Example 4: one epitope, many antigens
Selecting antibodies that bind to a specific pair or group of antigens is just as simple as finding the antibodies that distinguish between them. In this case, the target antigens can be used alternately to screen the library so that only antibodies recognizing both targets are isolated. This is both faster and more efficient than animal-based antibody generation, which usually relies on creating a peptide antigen representing a common protein sequence.

A choice of antibody formats
Antibodies from the HuCAL library are recombinant, which means that they can be very easily manipulated in vitro to create the ideal format for a variety of applications. The antibodies are initially screened in the form of monovalent Fab fragments. Once the desired monovalent antibody specificities are isolated, they can be cloned into a variety of antibody-expression vectors offering different formats and protein tags. For most applications, AbD Serotec recommends the use of bivalent Fab fragments. These have the advantage of increased avidity owing to the presence of two antigen binding sites, yet are considerably smaller than an immunoglobulin molecule, so they diffuse more readily and eliminate the cross-reactivity problems sometimes associated with the Fc region. The antibodies can also be tagged with Myc, His, Flag or Strep peptide sequences, or fused directly to recombinant enzymes such as alkaline phosphatase.

AgX® antigen expression service
HuCAL technology requires only about 0.5 mg of protein to generate antibodies, but even that amount is too high for some researchers. If the DNA sequence is available, peptides are often seen as the easiest choice as antigen. Antibodies generated against peptides, however, are often not ideal for use with the entire protein. The use of antigen fragments expressed as fusion proteins is an excellent alternative to peptides, as the fragments are more likely fold and thus present a more complex antigen for antibody selection. The AgX service from AbD Serotec includes advice on the selection of the fragment, and full-service cloning, expression and purification of the antigen.

Summary
HuCAL technology for rapid generation of highly specific monoclonal antibodies has many advantages over animal-based technologies. Not only is it considerably faster, it also offers direct selection of exquisitely specific antibodies in a variety of formats. The technology is entirely an in vitro system, so there are no animals involved at any stage of the procedure. To date, AbD Serotec has generated over 4,000 unique antibodies to more than 100 different antigens, with a greater than 90% success rate.


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