BIOTEM
Custom antibodies & services

RECOMBINANT ANTIBODY PLATFORM

PHAGE DISPLAY *Technology*

Project Strategy & Specifications

Target Typology

- Virus, bacteria, chemical compounds, haptens, etc.
- Proteins (intracellular, membrane, post-translational modifications, etc.)
- Peptides (design, synthesis and conjugation)

Final Applications

- ELISA, WB, IP, IF, IHC, FACS
- Diagnostic & Therapeutic (Human & Veterinary fields)

Initial Materials and Project Features

- Available material (quantity, purity)
- Immunogenicity / Toxicity
- Cross reaction and specificity

The phage display allows the generation of recombinant antibodies from naive or immune banks built from immunized animals (llamas, camels, dogs, cats, etc.).

Peptide Design & Synthesis

Immunization with peptides is a relevant strategy for the generation of antibodies directed against selected and specific epitopes. This approach also reduces the need for sophisticated protein preparations for immunization.

In order to obtain full project success, all the key parameters of peptide design must be taken into account:

Recombinant Proteins

BIOTEM has tailored solutions for the production of recombinant proteins as immunogen and antigen (soluble proteins, membrane proteins, GPCR, ion channels...).
Starting with a sequence and/or a plasmid cDNA, the target proteins can be obtained by exclusive methods in the purified form as part of proteo-liposomes and/or as soluble proteins.

Small Molecules / Haptens

Hapten conjugation strategy (hapten orientation, linker characteristics and hapten:carrier ratio) will greatly influence the quality of developed antibodies. BIOTEM disposes of **a chemical unit** dedicated to the custom development of immunogens: **Hapten synthesis**, **Hapten functionalization, Hapten conjugation** to carrier proteins and control (MALDI-TOF, NMR, LC/MS). Our expertise allows the development of optimized immunogens for the generation of antibodies with **high affinity** and **specificity**.

Custom Immunization

BIOTEM provides of a highly efficient service for the custom generation of recombinant monoclonal antibodies. Different protocols can be implemented individually or in combination. Extensive serum analysis (ELISA, affinity analysis, etc.) is performed. Tailored methodologies are developed to assess that appropriate immune responses have been achieved.

Several Species: Human & Veterinary Applications

An efficient and sophisticated technology, the Phage Display allows the generation of recombinant antibodies from naive or immune banks built from immunized animals. In addition to providing the ability to test a large number of clones thanks to the high throughput screening and access to nucleotide sequences early in the process, this technology allows the development of monoclonal antibodies all types of species

- **Llama, Camel & Non-Human Primate** (see Ultimate Humanization®)
- **Dog & Cat**
- **Cow, Horse, Goat, etc.**

The combination of a **strong natural immune response**, an **antigen based library construction and an optimized Phage Display screening** allows the generation of **high affinity antibodies** with a large epitope coverage.

Antibody Selection

A crucial step in the project to isolate reactive and specific clones. The methods used are adapted for each project according to the client's specifications. Several screening rounds are generally carried out. Successful antibody isolation by Phage Display technology depends on various factors such as strategies used for the library construction and for biopanning.

Phage Display Technology – Library Construction

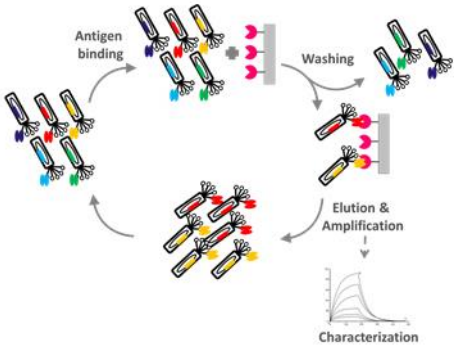
- B-cell isolation from the spleen, lymph node, PBMC or bone marrow
- Amplification by RT-PCR of the mRNA coding for the variable domains
- Construction of an hyper-immune library focus on the antigen (scFv or VHH)

Phage Display Technology – Biopanning

BIOTEM implements the best biopanning methods, adapted to the CLIENT's antigens:

- Direct
- Competitive
- Subtractive

Phage Display Biopanning

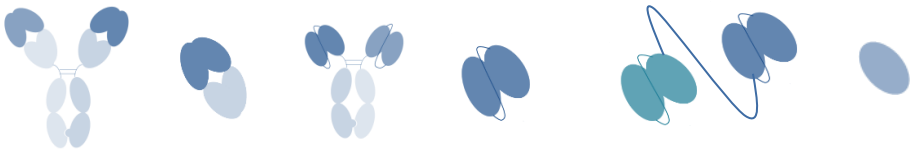


Post-panning isolated binders are individually selected, sequenced and tested for their reactivity / specificity by different methods (ELISA, affinity analysis, etc.).

The best clones will be produced (scFv or VHH format). Then, the CLIENT has the possibility to reformate the candidates (Full Ig).

Antibody Reformatting & Recombinant Ab Production

This step allows the transition from scFv / VHH (nanobody) to full immunoglobulin format thanks to a fully integrated platform for the production of recombinant antibodies in mammalian cells (CHO): **Several Isotypes, Sequence Optimization, Mutations, Fc-fusion Protein, Bispecific Antibodies, Fab**, etc.



Transient Transfection in CHO cells

- **Rapid & commonly used**
- **Serum-free culture** and **high yield** of production
- **Correct folding**, **assembly** and **post-translational modification**
- **Antibody Engineering** (Isotype, mutations, Fc-fusion protein, Bispecific antibodies, Fab, Chimerization, Humanization, etc.)

BIOTEM's Expertise

- ***Small & Large scale production:*** *From milligram to several grams*
- ***Optimized System: High yield and purity***
- ***« Low Bovine IgG »*** *and* ***« Low Endotoxin » conditions (< 10 EU/mg ; even < 1 EU/mg)***
- ***Quality Control & Antibody Characterization***

Purification – Downstream Process (DSP)

Depending on the physicochemicals characteristics of the antibody, the isotype and the origin (species, matrix, etc.), different purification strategies can be implemented. Our know-how and several proprietary protocols enable us to complete sucessfully the most difficult purifications (IgM, double isotype...)

- Affinity Chromatography: Protein A, G, A/G, L, Peptides or Proteins, etc.
- Ion Exchange or Size Exclusion Chromatography
- Precipitation (several methods)

Non-exhaustive list

Quality Control & Characterization

Spectrophotometry quantification, SDS-PAGE analysis, Endotoxin determination, ELISA, BLItz / Biacore, Stability studies, SEC-HPLC, DSC, etc.

BIOTEM

Parc d'Activités Bièvre Dauphine

885, rue Alphonse Gourju

38140 Apprieu – France

Tel : +33 (0)476 65 10 91

info@biotem.fr