BIOTEM
Custom antibodies & services

Rabbit MONOCLONAL ANTIBODIES

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)

Initial Materials and Project Features

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

Final Applications

- ELISA, WB, IP, IF, IHC, FACS
- Functional testing, quality controls
- Lateral Flow, etc.

Rabbit Monoclonal Antibodies achieve high affinity and specificity while allowing the recognition of a broad diversity of targets in Life Science and Diagnostics.

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 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.

Higher Specificity

Rabbits generate a highly diverse B-lymphocyte repertoire, especially through their CDR3-loop which is long when compared to other animals.

They are optimal for the detection of:

- **Small molecules** (toxins, pollutants, hormones, drugs, etc.)
- **Non-protein targets** (lipids, carbohydrates, etc.)
- **Post translational modifications** (phosphorylation, etc.)
- **Point mutations**

Higher Affinity

Thanks to a wider antibody repertoire and a more efficient affinity maturation system, rabbit antibodies frequently exhibit 10- to 100- fold higher affinity values than mice and other rodent monoclonal antibodies.

Affinities values (K_D) are often in the nanomolar (10^{-9} M) range and may go down to picomolar (10^{-12} M) range without any *in vitro* affinity maturation.

In addition, higher antibody affinities ensure higher signal-to-noise ratio when compared to mouse mAbs at a given antibody concentration.

Larger Epitope Coverage

Thanks to the long phylogenetic distance of rabbits (*Lagomorpha*) from humans and mice / rodents, human targets are often more immunogenic in rabbits than in mice.

Moreover the rabbit immune system presents different advantages:

- **Lower immune dominance, immune response is directed against various epitopes**
- **Larger B-cell repertoire**

In most cases novel epitope recognition is observed.

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.

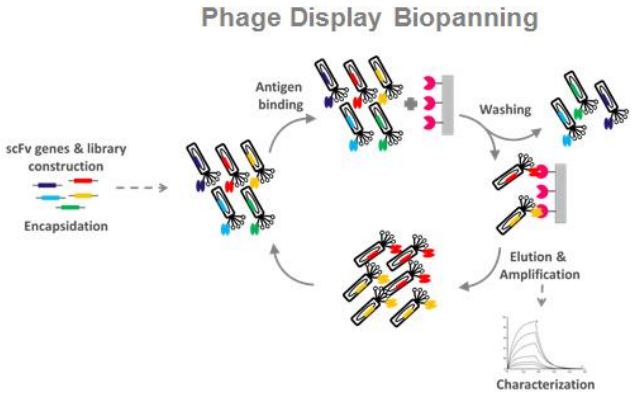
Phage Display Technology – Library Construction

- B-cell isolation from the spleen
- Amplification by RT-PCR of the mRNA coding for the variable domains VLκ, VLλ and VH
- Construction of a scFv hyper-immune library focus on the antigen

Phage Display Technology – Biopanning

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

- Direct
- Competitive
- Subtractive



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 selected for the reformatting phase and the production.

Antibody Reformatting & Recombinant Ab Production

This step allows the transition from scFv 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 physicochemical 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 successfully 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.

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