BIOTEM
Custom antibodies & services

Mouse & Rat MONOCLONAL ANTIBODIES

HYBRIDOMA & PHAGE DISPLAY *Technologies*

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
- Functional testing, quality controls
- Lateral Flow, etc.

Initial Materials and Project Constraints

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

BIOTEM has developed a large panel of improved strategies enabling us to complete the most challenging projects.

Peptide Design

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

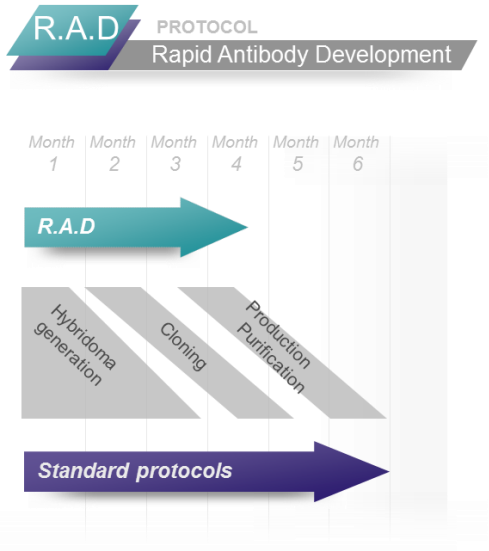
BIOTEM has tailored solutions for the production of recombinant proteins as immunogen and antigen (soluble proteins, membrane proteins, GPCR, ion channels...).
Starting with a sequence and/or a plasmid cDNA, the target proteins can be obtained by exclusive methods in the purified form as part of proteo-liposomes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



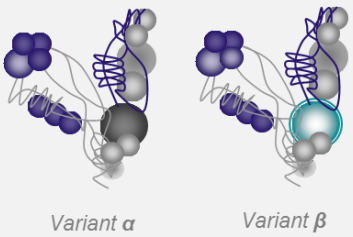
R.A.D® Protocol — Exclusiveness BIOTEM

This exclusive method allows the fast development of hybridomas in less than a month (immunization, fusion and screening included). The *R.A.D® Protocol* therefore enables us to shorten by up to 3 months the duration of complete projects (including the stages of cloning and the production/purification of antibodies).

- **Rapidity** (<1 month)
- **Conserved Targets**
- **High Hybridoma Yield**

Subtractive Immunization

BIOTEM masters various subtractive immunization methods. These techniques are particularly useful for the generation of antibodies against epitopes that are poorly immunogenic and/or sharing a high degree of homology.



S.S.I Protocol

Due to the small size of some proteins we strongly recommend to implement our *S.S.I Protocol* (Small Size Immunogen).

This technology uses a specific immunization protocol with a mixture of free and conjugated immunogen.

In Vitro Immunization

The injection of an immunizing agent isn't always the most pertinent approach to develop monoclonal antibodies.

Some molecules are indeed very toxic after injection and/or not immunogenic.

In order to circumvent some of the inherent problems with *in vivo* immunization, BIOTEM can propose alternative solutions based on *in vitro* immunization protocols.

Standard & High Affinity Protocol (Kohler & Milstein)**, Genetic Immunization, Surface Epitope Masking** (SEM)**,** etc.

Following an in-depth analysis of the immune responses, BIOTEM selects the best animals in order to build highly representative hyper-immune libraries focused on the antigen.

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 (standard, competitive, multiple screening, etc.). Several screening rounds are generally carried out.

Hybridoma Technology – Fusion

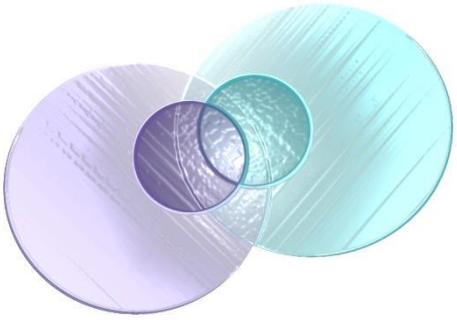
- Lymphocyte hybridization with myeloma cells
- Plating out of fused cells on microtitre plates (96 wells)
- Culture in selective medium

Hybridoma Technology – Screening

- Primary screening (polyclonal stage)
- Confirmation (polyclonal stage)
- Several methods available: ELISA, Western Blot, Immunohistochemistry, etc.
- Direct, Competitive, Subtractive, etc.

Hybridoma Technology – Cloning

- Hybridoma cloning by limiting dilutions (monoclonal stage)
- Selection of sub-clones
- Validated cell bank with viability evaluation



The development of monoclonal antibodies includes several key screening stages. BIOTEM supports the development and the selection of hybridomas via optimized screening/cloning techniques.

Phage Display Technology – Library Construction

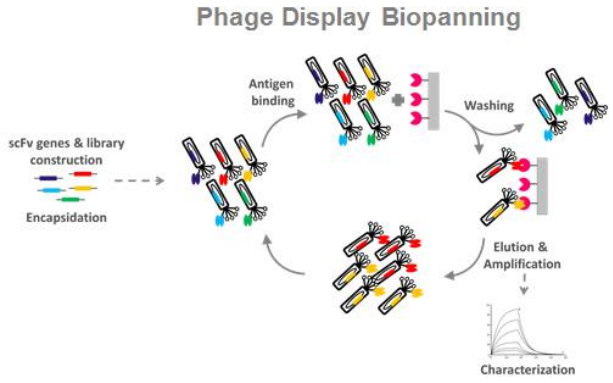
- B-cell isolation from the spleen
- Amplification by RT-PCR of the mRNA coding for the variable domains VLκ, VLλ and VH
- Construction of a scFv hyper-immune library focus on the antigen

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, …).



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

Antibody Reformatting (for Phage Display or recombinant Ab)

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.

Antibody Production & Purification

BIOTEM offers different solutions for the production and purification of antibodies, from pilot batch (milligrams) to large-scale (several grams).

In vitro Production – Upstream Process (USP)

From Hybridomas

Low density production

- Flask
- HYPERFlask®

High density production

- Membrane based CELLine™
- Hollow fiber Bioreactors (FiberCell System)

From Recombinant Antibody

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***

In vivo Production (ascite)

From *nude* and *BALB/c* mice

Nude mice's immunodeficiency allows them to develop hybridomas from other species (human, rat, chimerical, etc.).
The ascitic fluid production enables the obtention of high yields of antibody.

Available only for Hybridomas.

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

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