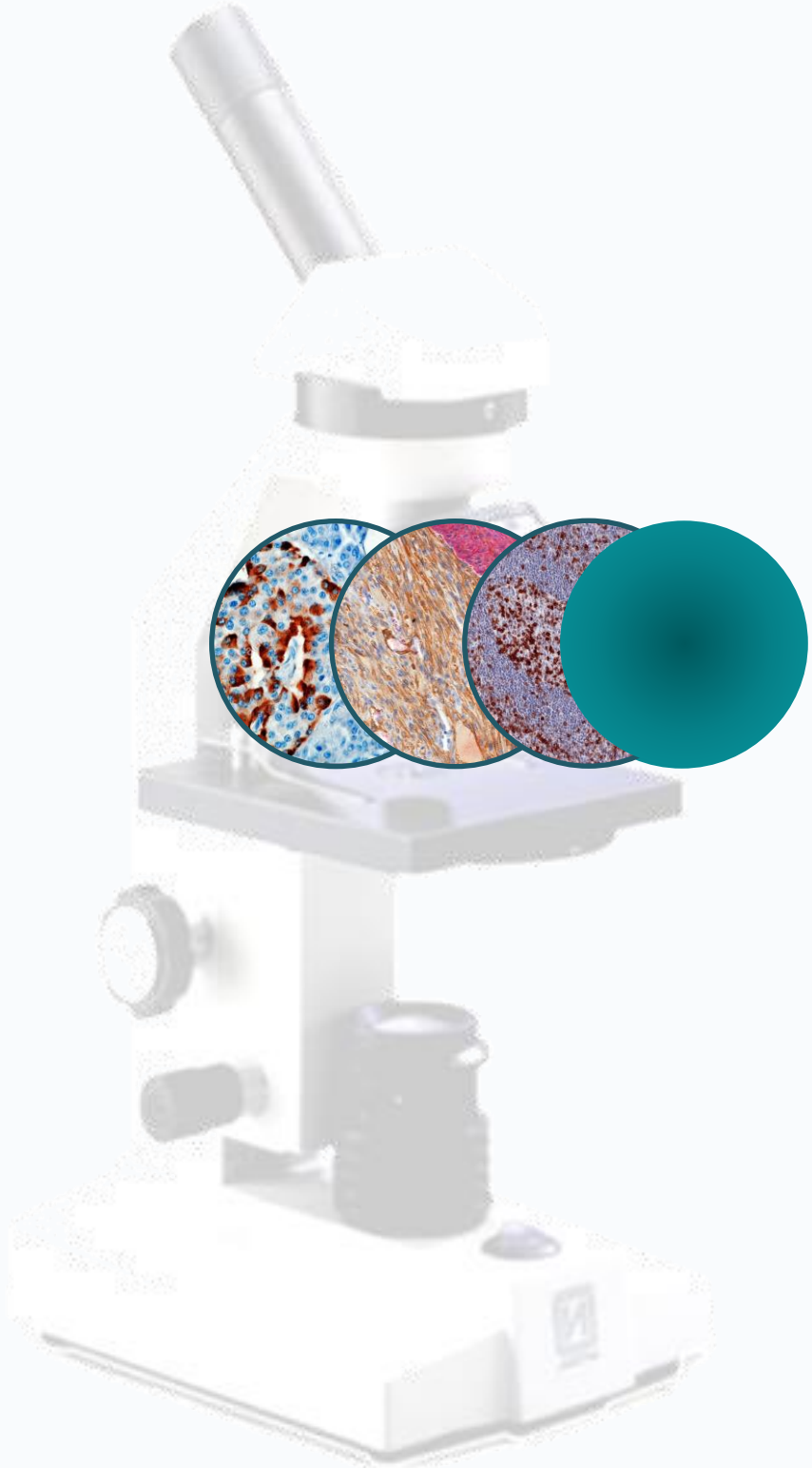
BIOTEM
Custom antibodies & services



IMMUNOHISTOCHEMISTRY 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)

Initial Materials and Project Constraints

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

Applications: Immunohistochemistry (IHC)

- Frozen sections
- Paraffin-embedded sections (IHC-P, formalin-fixed, paraffin-embedded slides or FFPE slides)

BIOTEM offers a platform entirely dedicated to the development of functional monoclonal antibodies in IHC with a particularly high success rate for paraffin-embedded samples.

Exclusiveness BIOTEM

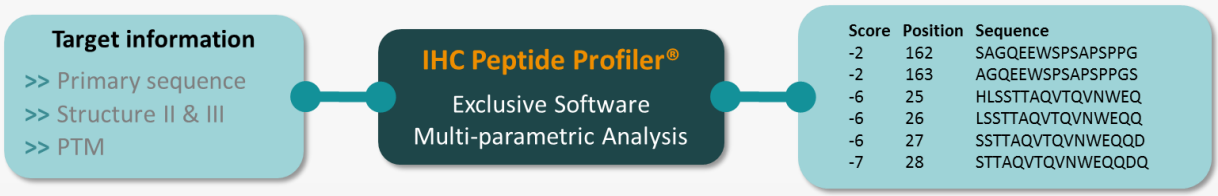
IHC Peptide Profiler®

To address the challenge of IHC-P antibodies, BIOTEM has developed an **exclusive software** called the **IHC Peptide Profiler®**. This software has been designed to generate **first-class peptides** to be used as immunogens in IHC-P antibody projects. Peptides generated by the software are fully suitable with any downstream technologies (Hybridoma and/or Phage Display).

The software integrates complex algorithms which allow to sort out optimal peptides in a 3-steps process:

1- **Modelisation of target altered structure in the FFPE environment**
2- **Primary peptide design**
3- **Final peptide selection and ranking**

All peptides generated during the primary design are further analyzed and ranked based on specific IHC-P criteria as well as more conventional and applicable parameters such as antigenicity, immunogenicity and solubility. The results can be extracted and summarized in a user friendly file which allows convenient selection of the final peptide candidates.



IHC Protein Formulations

Available only from BIOTEM, the IHC-specific antigen formulations that are designed to subtly modify proteins in order to obtain immunogens that mimic the targeted epitopes as they appear on IHC paraffin sections. Such a complementary approach to the IHC Peptide Profiler® allows a broadening of the eventual diversity in generated antibodies.

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 (IHC, ELISA, affinity analysis, etc.) is performed. Tailored methodologies are developed to assess that appropriate immune responses have been achieved.

- **Mouse & Rat Monoclonal Antibody** (**Hybridomas** or **Phage Display**)
- **Rabbit Monoclonal Antibody** (**Phage Display**)
- **Other Species** (**Phage Display**)

Antibody Selection

Hybridoma Technology – Fusion

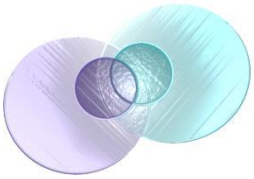
- Lymphocyte hybridization with myeloma cells
- Plating out of fused cells on microtitre plates (96 wells)
- Culture in selective medium

Hybridoma Technology – Screening

- Primary screening (polyclonal stage)
- Confirmation (polyclonal stage)
- Several methods available: Immunohistochemistry, ELISA, Western Blot, 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

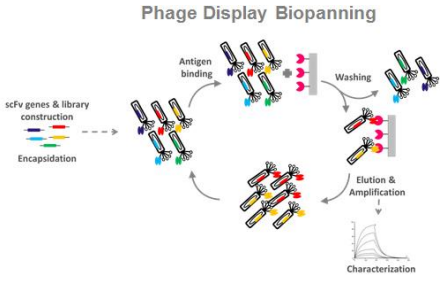
- B-cell isolation from the spleen
- Amplification by RT-PCR of the mRNA coding for the variable domains VLκ, VLλ and VH
- Construction of a scFv hyper-immune library focus on the antigen

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 (IHC, 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 Hybridomas

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.

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

BIOTEM

Parc d'Activités Bièvre Dauphine

885, rue Alphonse Gourju

38140 Apprieu – France

Tel : +33 (0)476 65 10 91

info@biotem.fr