ProteoGenix

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Free Whitepaper

ANTIBODY PRODUCTION MADE SIMPLE: A CLEAR AND CONCISE GUIDE

Essential insights into antibody generation methods, applications, challenges, and future trends

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Introduction

Antibodies play a crucial role in the immune system, identifying and neutralizing foreign substances such as bacteria and viruses.

The unique properties inspired researchers to exploit them in biomedicine, and they are now widely used in diagnostic and therapeutic applications. These applications have been made possible thanks to the development of advanced antibody generation and production techniques.

This whitepaper aims to describe the fundamentals of antibody production, revisiting the concepts of monoclonal and polyclonal antibodies, detailing the methods of antibody generation and production, and exploring the applications, challenges, and future perspectives of these biomolecules.

Among these future trends, the growing importance of artificial intelligence in antibody development stands out. This guide also explores the key contributions of AI, highlighting how it is transforming the way antibodies are engineered.

To close, we present a benchmark success story from our work with Telum Therapeutics—demonstrating how advanced antibody engineering can translate into impactful therapeutic outcomes.

What is Antibody Production?

Antibody production is a vital, multifaceted process crucial for both natural immunity and therapeutic applications. Understanding this process involves exploring different contexts of antibody generation:

1. In Vivo Antibody Production

In vivo antibody production is the natural immune response to antigens. Key steps include:

- Antigen Presentation: Dendritic cells present antigens to T-helper cells.
- B Cell Activation: T-helper cells activate B cells.
- Antibody Secretion: B cells become plasma cells and secrete antibodies.
- Memory Formation: Some B cells become memory cells for longterm immunity.

2. Antibody Production as a Biotechnological Process

This industrial process involves:

- Antigen preparation
- Screening and selection
- Cloning and expression
- Purification and characterization

Controlling the entire process from gene design to antibody generation offers significant technical advantages. It ensures optimal sequence design for expression and stability, guarantees data integrity across all stages, and enables full traceability of the molecule's development. This integrated approach facilitates troubleshooting, accelerates iteration, and ultimately leads to more robust and reproducible antibodies.

3. Recombinant Antibody Production

Recombinant antibody production uses genetic engineering to produce antibodies without animal immunization

Steps include:

- Gene isolation and cloning: inserting antibody genes into expression vectors
- Expression in host cells: using bacteria, yeast, or mammalian cells.
- Culturing and harvesting: growing and harvesting the host cells
- Purification: Obtaining high-quality antibodies.

This method allows for greater control and scalability in antibody production.

Each expression system has its own advantages and limitations. At ProteoGenix, we offer no fewer than five different systems to address a wide range of production challenges.

ProteoGenix Transient Expression Handbook

To learn more about recombinant protein expression, we invite you to explore our dedicated eBook on the topic.

DISCOVER

Polyclonal Antibody Production

Polyclonal Antibody Production is a vital technique in immunology and biotechnology, widely used in diagnostics and research.

WHAT ARE POLYCLONAL ANTIBODIES ?

Polyclonal antibodies (pAbs) are a mixture of antibodies from different B cell clones, recognizing multiple epitopes on a single antigen. This makes them versatile and effective for detecting various antigen forms. Polyclonal Antibody Production is a vital technique in immunology and biotechnology, widely used in diagnostics and research.

WHAT ANIMALS ARE USED FOR THEIR PRODUCTION ?

Mice and Rats: Suitable for small scale production and research.

Rabbits: High-affinity antibodies, ideal for diagnostics.

Goats and Sheep: Used for large scale antibody production.

Chickens: Produce IgY antibodies in egg yolk, useful for avoiding mammalian cross-reactivity.

Monoclonal antibody generation

Monoclonal antibodies (mAbs) have transformed the fields of biotechnology, diagnostics, and therapeutics with their remarkable specificity and consistency. Today, monoclonal antibodies (mAbs) account for over half of all new biologic approvals. The global therapeutic antibody market projected to reach over 450 billion USD by 2030 *.

In this segment, we delve into the definition of monoclonal antibodies, production techniques, as well as the pros and cons associated with each method.

WHAT ARE MONOCLONAL ANTIBODIES?

Monoclonal antibodies are uniform antibodies generated by a single clone of B cells. In contrast to polyclonal antibodies, which consist of a blend of antibodies from various B cells, monoclonal antibodies identify and attach to a singular specific epitope on an antigen. This exceptional specificity renders monoclonal antibodies as precise instruments for aiming at particular molecules, offering numerous significant benefits:

- Specificity: Monoclonal antibodies bind to one specific epitope on an antigen, reducing the risk of cross-reactivity and non-specific binding.
- Consistency: As they are produced from a single clone of cells, monoclonal antibodies provide uniformity in their structure and function, ensuring consistent performance across different batches.
- Versatility: They can be engineered and modified for various applications, including diagnostic tests, therapeutic treatments, and research tools.
- Therapeutic Potential: Due to their ability to specifically target disease-related molecules, monoclonal antibodies are widely used in treating conditions such as cancer, autoimmune diseases, and infectious diseases.

* Ref: https://www.bioprocessonline.com/doc/a-look-at-the-growing-market-landscape-for-monoclonal-antibodies-0001

Methods for Generating Monoclonal Antibodies In Vivo

Hybridoma Technology

Hybridoma technology is an in vivo approach used to produce monoclonal antibodies by merging antibody-producing B cells from an immunized animal with myeloma cells. This fusion creates hybrid cells that can be cloned and expanded to generate large quantities of identical antibodies.

+

PROS OF HYBRIDOMA TECHNOLOGY:

- High specificity and affinity of antibodies
- Stability of hybridoma cell lines for long-term antibody production
- Well-established and commonly used in the field

−

LIMITATIONS OF HYBRIDOMA TECHNOLOGY:

- Time-consuming and labor-intensive
- Ethical concerns regarding animal use
- Restricted to producing antibodies from specific species like mice or rats
- Need for transgenic mice or humanization process for human antibodies
- Risk of myeloma cell contamination impacting antibody quality

Single B Cell Sorting

Single B cell sorting is an in vivo technique used to create monoclonal antibodies. It involves isolating and cloning individual B cells that generate particular antibodies. These B cells are usually sourced from immunized animals or humans and are categorized by their antigen specificity.

+

PROS OF SINGLE B CELL SORTING:

- High specificity and affinity of antibodies
- Rapid identification and cloning of antigen-specific B cells
- Suitable for human antibodies. No need for lengthy humanization processes

−

LIMITATIONS OF SINGLE B CELL SORTING:

- Requires access to immunized individuals or animals
- Technically demanding and needs specialized equipment
- Limited number of isolated antigen-specific B cells
- Human antibodies from animals may need humanization for therapeutic use

Methods for Generating Monoclonal Antibodies In Vivo

Phage Display

Phage display is a technique used to create monoclonal antibodies in vitro. It involves showcasing antibody fragments on bacteriophages' surfaces (viruses that infect bacteria). This method enables quick screening and selection of high-affinity antibodies from extensive libraries of antibody fragments.

+

PROS OF PHAGE DISPLAY TECHNOLOGY:

- Ethical antibody production without animal use
- Rapid generation and screening of large antibody libraries
- High flexibility in engineering and modifying antibodies
- No species restriction for antibody derivation
- Adaptability for diverse applications, including developing antibodies against challenging antigens.

−

LIMITATIONS OF PHAGE DISPLAY TECHNOLOGY:

- Requires sophisticated infrastructure and expertise
- Antibodies may have lower affinity
- Limited complexity of antigens
- Restrictions on antibody development.

Choosing the right approach requires in-depth expertise to align the strengths of each method with the specific goals and constraints of a given project. At ProteoGenix, we believe it is our responsibility to recommend the most suitable strategy — not in a one-size-fits-all manner, but through thoughtful discussions with our clients to fully understand their needs and expectations. We have all the methods described in this e-book at our disposal, allowing us to match each project with the most effective solution — whatever the complexity of the challenge.

Differences Between Polyclonal and Monoclonal Antibodies

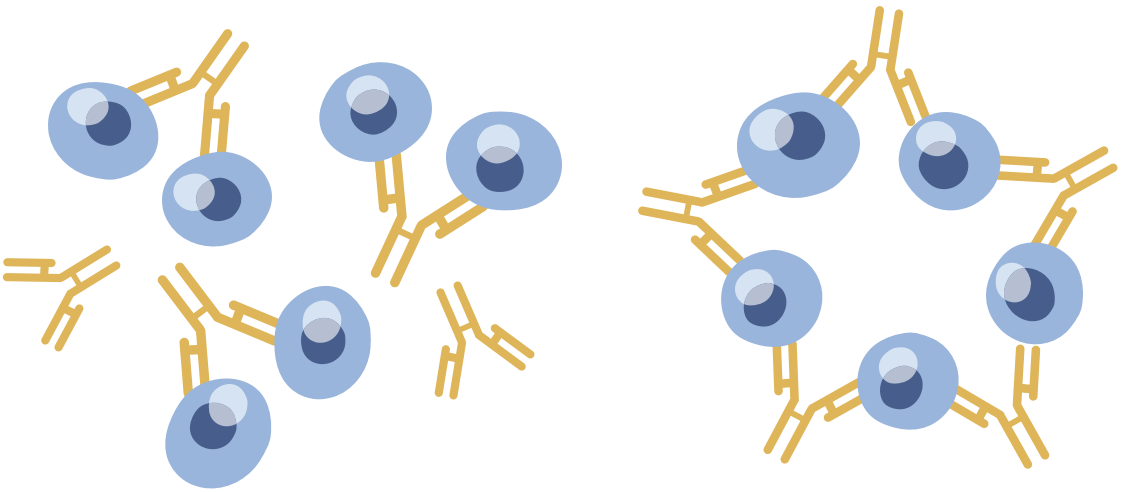
What are the differences between polyclonal and monoclonal antibodies ?

POLYCLONAL ANTIBODIES:

Polyclonal antibodies (pAbs) are produced by multiple B cell clones, resulting in a heterogeneous mixture of antibodies that recognize and bind to various epitopes on a single antigen. This polyclonal nature makes them highly versatile and capable of detecting different forms and variations of the target antigen, which can be particularly useful in applications where a broad detection range is desired.

MONOCLONAL ANTIBODIES:

Monoclonal antibodies (mAbs) are produced by a single B cell clone, resulting in a homogeneous population of antibodies that recognize and bind to a single, specific epitope on the antigen. This specificity makes monoclonal antibodies highly precise tools for targeting and neutralizing specific molecules. The consistent and reproducible nature of monoclonal antibodies makes them ideal for therapeutic applications, where uniformity and high specificity are critical. Additionally, monoclonal antibodies are less likely to exhibit cross-reactivity compared to polyclonal antibodies.



MONOCLONAL

POLYCLONAL

Main characteristics of pAbs and mAbs

	Polyclonal Antibodies (pAbs)	Monoclonal Antibodies (mAbs)
Production Source	Produced by multiple B cell clones	Produced by a single B cell clone
Heterogeneity	Heterogeneous mixture - recognizes various epitopes on a single antigen	Homogeneous - recognizes a single specific epitope on the antigen
Versatility	Highly versatile, capable of detecting different forms and variations of the target antigen	Highly specific, precise targeting and neutralization of specific molecules
Detection Range	Broad detection range, useful in applications requiring detection of different antigen forms and variations.	Narrow detection range – focused on a single epitope
Detection Limit	Low detection limit - advantageous for various diagnostic and research applications	Higher detection limit compared to polyclonal antibodies
Batch Consistency	Potential batch-to-batch variability due to heterogeneous nature	High consistency and reproducibility between batches due to homogeneous nature
Cross-Reactivity	Potential for cross-reactivity with non-target antigens	Less likely to exhibit cross-reactivity
Therapeutic Suitability	Not suitable for therapeutic applications due to lack of reproducibility	Ideal for therapeutic applications - requiring high specificity and reproducibility
Production Complexity	Easy and fast to produce	More complex and time-consuming to produce
Cost	-	+
Application Suitability	Useful in diagnostic and research applications requiring broad antigen detection and low detection limits	Suitable for therapeutic applications and research requiring precise targeting and consistent results

Monoclonal antibody production

Antibody production refers to the entire process of producing an antibody with specificity to a distinct biological target.

Ascites production

Ascites production is an in vivo method that involves injecting hybridoma cells into the peritoneal cavity of a mouse. The hybridoma cells produce antibodies that accumulate in the ascitic fluid, which is then harvested.

PROS OF ASCITES PRODUCTION TECHNOLOGY:

- High antibody yield in a short time frame
- Useful for rapid large-scale antibody production
- Antibodies in ascites fluid often have high titers and are easily purified

LIMITATIONS OF ASCITES PRODUCTION TECHNOLOGY:

- Ethical concerns due to animal usage
- Variability in antibody quality and yield based on animal health
- Risk of contamination with mouse proteins and other substances, complicating purification and impacting antibody application in clinical or diagnostic scenarios.

Hybridoma Cell culture

Hybridoma cell culture is an in vitro method where hybridoma cells, created by fusing B cells from an immunized mouse with myeloma cells, are cultured in a controlled environment to produce antibodies. The hybridoma cells can be grown in various culture systems, including flasks, bioreactors, or other large-scale production systems.

+

PROS OF HYBRIDOMA CELL CULTURE:

- High specificity and affinity
- Consistent and controlled production
- Establishment of stable hybridoma cell lines for continuous antibody supply
- Ethical concerns related to animal use in ascites production are eliminated.

−

HYBRIDOMA CELL CULTURE LIMITATIONS INCLUDE:

- Time-consuming.
- Genetic instability leading to variations in antibody production.
- Requires significant resources and expertise, which can be costly for research facilities.

Recombinant Antibody production

Recombinant antibody production using DNA technology has become a cornerstone in the field of biopharmaceuticals, offering precise and scalable methods to generate monoclonal antibodies. This section includes a definition of recombinant antibody production, descriptions of transient and stable antibody expression, and an overview of commonly used expression systems.

What is Recombinant Antibody production?

Recombinant antibody production involves the use of genetic engineering to create antibodies in host cells. This process entails inserting the genes encoding the desired antibody into an expression system, which then produces the antibody. This method allows for high specificity and control over antibody characteristics, making it highly suitable for therapeutic, diagnostic, and research applications. Recombinant technology enables the production of antibodies with consistent quality and the possibility of tailoring antibody features such as affinity and specificity.

TRANSIENT AND STABLE ANTIBODY EXPRESSION

In the context of recombinant antibody production, there are two primary approaches: transient and stable expression.

Transient Antibody Expression

Transient expression involves temporarily introducing plasmid DNA encoding antibodies into host cells, leading to short-term antibody production

Advantages

- Rapid production for short-term studies without the need for stable cell lines.

Limitations

- Include the need for frequent transfections and lower yield compared to stable systems.

Stable Antibody Expression

Stable expression integrates antibody genes into host cell genome for continuous antibody production. Process involves transfection, cell selection, and cloning to establish stable cell lines.

Advantages

- Long-term, high yield production suitable for large-scale manufacturing.

Limitations

- Requires complex, costly setup but offers efficiency and reliability compared to transient expression.

Main characteristics of Transient and Stable Antibody Expression

	Transient Antibody Expression	Stable Antibody Expression
Expression Method	Temporary introduction of plasmid DNA into host cells	Integration of antibody genes into the host cell genome
Duration	Short-term (few days to weeks)	Long-term and continuous
Production Timeline	Rapid production, suitable for short-term studies and early-stage research	Time-consuming initial setup
Quantity Produced	Small to moderate quantities	High yield and scalability
Applications	Early-stage research, proof-of-concept studies, small-scale production	Large-scale manufacturing, therapeutic antibody production, consistent and reliable antibody supply
Advantages	Rapid production timeline; suitable for producing small to moderate quantities without stable cell lines	Long-term consistent production, high yield, scalable for large-scale manufacturing
Limitations	Transient nature of expression; lower yield and consistency compared to stable systems	Time-consuming and labor-intensive to establish; requires a complex and costly initial setup

The Ultimate Stable Cell Line Development Guide

To learn more about stable cell line development, you can read our dedicated ebook on the topic.

DISCOVER

EXPRESSION SYSTEMS FOR ANTIBODY PRODUCTION

Various expression systems are utilized for producing recombinant antibodies, each offering specific benefits and constraints. The commonly utilized systems comprise bacterial and mammalian expression systems:

Bacterial Expression Systems

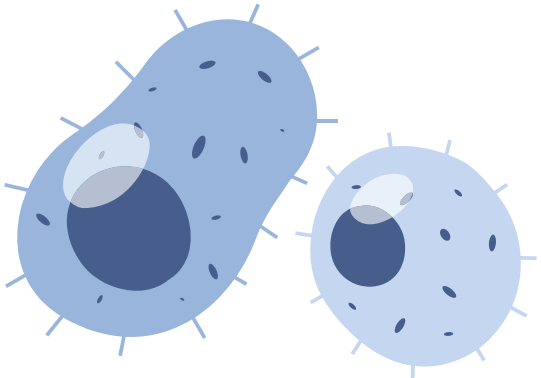
Bacterial systems, particularly Escherichia coli (E. coli) and Bacillus subtilis, are popular for antibody production due to their rapid growth and high-density cultures, leading to high productivity.

Advantages

- Cost-effective cultivation process.
- Straightforward, with well-characterized genetics.
- Wide range of molecular biology tools available.

Limitations

- Limited in producing full-length antibodies with essential post-translational modifications.
- Complex antibody structures can face protein folding and solubility challenges in bacterial systems.



Mammalian Expression Systems

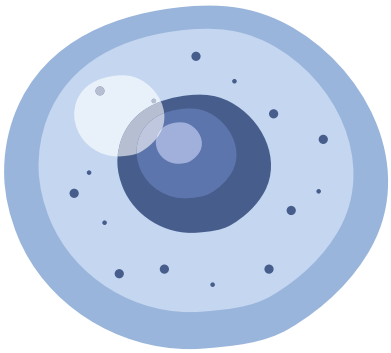
CHO and HEK 293 cells are chosen for antibody production as they produce full-length antibodies with important post-translational modifications like glycosylation, ensuring therapeutic antibodies' effectiveness and stability.

Advantages

- High expression levels and scalability, particularly in CHO cells.
- Ideal for large-scale production.
- Essential for producing a wide range of antibody formats, (full-length IgG antibodies, bispecific antibodies, and Fc-fusion proteins).

Limitations

- More complex and costly to cultivate.
- Longer development time.
- Require more sophisticated infrastructure and expertise.



Main characteristics of Bacterial and Mammalian Antibody Expression Systems

	Bacterial Expression Systems	Mammalian Expression Systems
Growth and Cultivation	Rapid growth and high-density cultures	More complex to cultivate
Productivity	High productivity	High expression levels and scalability
Cost	Cost-effective and straightforward	More costly
Genetic Tools	Well-characterized genetics and wide range of molecular biology tools available	Requires more sophisticated genetic tools
Antibody Types Produced	Primarily smaller antibody fragments like scFvs and Fab fragments	Full-length IgG antibodies, bispecific antibodies, and Fc- fusion proteins
Post-Translational Modifications	Limited ability for proper post-translational modifications such as glycosylation	Capable of proper post-translational modifications, including glycosylation
Protein Folding/ Solubility	Protein folding and solubility issues with complex antibody structures	Better handling of protein folding and solubility
Applications	Suitable for research, diagnostics, and certain therapeutic applications	Suitable for therapeutic applications requiring full-length antibodies

HOW TO CHOOSE BETWEEN THESE TWO EXPRESSION SYSTEMS?

Recombinant antibody production using DNA technology is a versatile and scalable approach for generating monoclonal antibodies. The choice between transient and stable expression methods depends on the desired production scale and timeline. Bacterial systems are ideal for producing smaller antibody fragments quickly and cost-effectively, while mammalian systems are necessary for producing fully functional antibodies with the appropriate modifications for therapeutic use. Understanding the advantages and limitations of each method and system enables researchers and manufacturers to select the most suitable approach based on their specific requirements.

Applications of Monoclonal Antibodies

Antibody production has transformed therapeutics, diagnostics, and research by utilizing antibodies' unique properties for treating diseases, creating diagnostic tools, and enhancing scientific knowledge.

Therapeutics | Diagnostics | Research

Therapeutics application

WHAT IS THE ROLE OF MABS IN THERAPEUTICS APPLICATIONS?

In therapeutics, monoclonal antibodies (mAbs) are employed to treat a wide range of diseases, including cancers, autoimmune disorders, and infectious diseases. Therapeutic antibodies can be designed to target specific antigens on cancer cells, marking them for destruction by the immune system or directly inhibiting their growth. Examples include rituximab for lymphoma and trastuzumab for breast cancer. Additionally, antibodies are used to modulate immune responses in conditions like rheumatoid arthritis and multiple sclerosis by targeting specific cytokines or cell surface receptors.

WHAT ARE THE CHALLENGES IN PRODUCING THERAPEUTIC ANTIBODIES?

One of the significant challenges in therapeutic antibody production is the risk of immunogenicity, where the patient's immune system recognizes the antibody as foreign and mounts an immune response against it. This issue can be mitigated by using humanized or fully human antibodies produced through transgenic mice or human phage display libraries. Another challenge is achieving high yield and purity, which is crucial for therapeutic applications. Solutions include optimizing cell culture conditions, using high-efficiency expression systems, and employing advanced purification techniques.

Cancer

Monoclonal antibodies have been transformative in oncology , offering targeted treatments that can specifically bind to cancer cells while sparing normal tissues. Unlike classical chemotherapy, which often affects both cancerous and healthy cells, leading to significant side effects, monoclonal antibodies provide a more precise approach. Commonly targeted cancers include breast cancer, lymphoma, leukemia, and colorectal cancer.

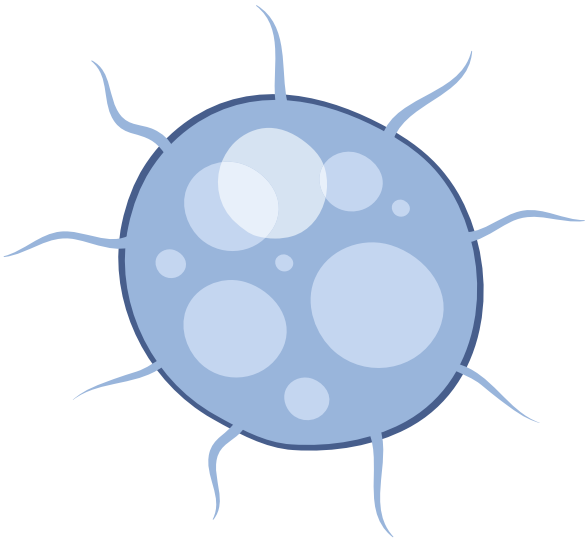
COMMON CANCER TARGETS

- **HER2/neu:** Trastuzumab (Herceptin) targets the HER2/neu receptor, which is overexpressed in some breast cancers. By binding to this receptor, trastuzumab inhibits the proliferation of cancer cells and induces antibody-dependent cellular cytotoxicity (ADCC).

- **CD20:** Rituximab (Rituxan) targets CD20, a protein expressed on the surface of B-cells. This antibody is used to treat non-Hodgkin lymphoma and chronic lymphocytic leukemia (CLL) by inducing cell lysis through ADCC and complement-dependent cytotoxicity (CDC).

- **EGFR:** Cetuximab (Erbitux) targets the epidermal growth factor receptor (EGFR), which is overexpressed in various cancers, including colorectal cancer and head and neck cancers. By blocking EGFR signaling, cetuximab inhibits tumor growth and proliferation.

- **VEGF:** Bevacizumab (Avastin) targets vascular endothelial growth factor (VEGF), a key regulator of angiogenesis. By inhibiting VEGF, bevacizumab reduces the blood supply to tumors, thereby inhibitingtheir growth.



Autoimmune Diseases

Monoclonal antibodies are used to treat autoimmune diseases by targeting specific components of the immune system that are involved in the pathological immune response. Commonly treated conditions include rheumatoid arthritis, multiple sclerosis, and psoriasis.

COMMON AUTOIMMUNE DISEASES TARGETS

- **TNF-α:** Infliximab (Remicade) and adalimumab (Humira) target tumor necrosis factor-alpha (TNF-α), a cytokine involved in systemic inflammation. By neutralizing TNF-α, these antibodies reduce inflammation and halt disease progression in rheumatoid arthritis, psoriasis, and inflammatory bowel disease (IBD).
- **IL-6 Receptor:** Tocilizumab (Actemra) targets the interleukin-6 (IL-6) receptor, blocking IL-6 signaling. This action is beneficial in treating rheumatoid arthritis by reducing inflammation and joint damage.
- **CD20:** Ocrelizumab (Ocrevus) targets CD20 and is used in the treatment of multiple sclerosis. By depleting B-cells, it helps in reducing disease activity and progression.

Infectious Diseases

Monoclonal antibodies have also shown promise in the treatment and prevention of infectious diseases. They can provide passive immunity by targeting specific pathogens or their toxins.

COMMON TARGETS

RSV F Protein: Palivizumab (Synagis) targets the fusion protein of the respiratory syncytial virus (RSV). It is used as a prophylactic treatment to prevent RSV infection in high-risk infants.

Clostridium difficile Toxin B: Bezlotoxumab (Zinplava) targets toxin B produced by Clostridium difficile, reducing the recurrence of C. difficile infections by neutralizing the toxin.

Diagnostics

WHAT IS THE ROLE OF MABS IN DIAGNOSTICS APPLICATIONS?

Antibodies are indispensable tools in diagnostics due to their high specificity and affinity for their target antigens. They are used in various diagnostic assays, such as enzyme-linked immunosorbent assays (ELISA), lateral flow assays (such as pregnancy tests), and immunohistochemistry. These assays can detect and quantify specific proteins, pathogens, or biomarkers in biological samples, aiding in disease diagnosis, monitoring, and research.

WHAT ARE THE CHALLENGES IN DIAGNOSTIC ANTIBODY PRODUCTION?

A common challenge in diagnostic antibody production is ensuring batch-to-batch consistency and high sensitivity. This can be addressed by using recombinant antibody production methods, which provide greater control over antibody characteristics and reproducibility. Additionally, antibodies used in diagnostics must exhibit high stability and resistance to environmental variations, which can be improved through protein engineering and selection of robust antibody formats.

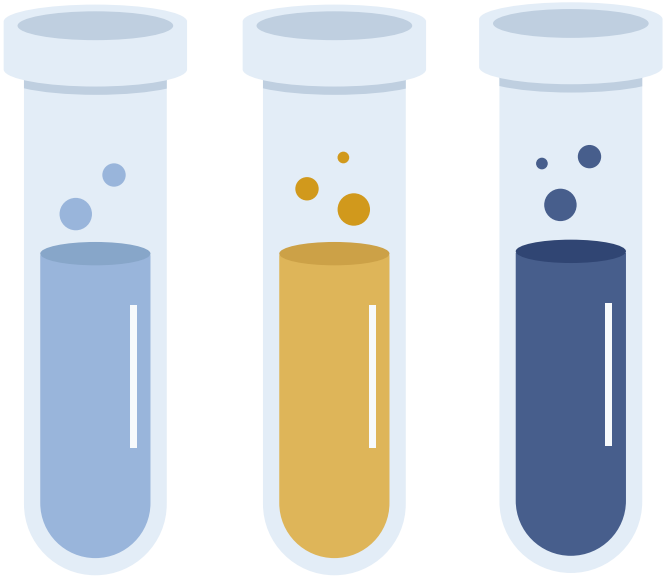
Research

WHAT IS THE ROLE OF MABS IN RESEARCH APPLICATIONS?

In research, antibodies are essential for a variety of applications, including Western blotting, flow cytometry, immunoprecipitation, and microscopy. They allow scientists to study the presence, quantity, and function of proteins within complex biological systems, contributing to our understanding of cellular processes and disease mechanisms.

WHAT ARE THE CHALLENGES IN USING ANTIBODIES FOR RESEARCH APPLICATIONS?

Research applications often require antibodies with specific characteristics, such as high affinity and minimal cross-reactivity. Producing such antibodies can be challenging, especially for targets that are highly conserved or poorly immunogenic. Hybridoma technology and phage display libraries offer solutions by enabling the selection of high-affinity antibodies against a wide range of antigens. Additionally, recombinant antibody technologies allow for the rapid production and modification of antibodies to meet specific research needs.



Challenges and solutions in antibody production

The production of monoclonal antibodies, while highly beneficial, presents several technical and logistical challenges. These include immunogenicity, low yield, post-translational modifications, immunogenic glycosylation profiles, aggregation, and stability.

- Immunogenicity
- Low yield
- Post-translational modifications
- Immunogenic glycosylation profiles
- Aggregation issues and stability concerns

Immunogenicity

How to address immune responses when using therapeutic antibodies?

USING HUMANIZED OR FULLY HUMAN ANTIBODIES

Therapeutic antibodies can sometimes trigger immune responses in patients, particularly when they contain non-human sequences. This challenge is addressed by using humanized or fully human antibodies. Humanization involves modifying a non-human antibody (typically derived from a mouse) to make its protein sequence more similar to human antibodies. This is done by grafting the non- human antibody's complementarity-determining regions (CDRs), which are responsible for antigen binding, onto a human antibody framework.

TRANSGENIC MICE AND PHAGE DISPLAY LIBRARIES

Additionally, transgenic mice that express human immunoglobulin genes and human phage display libraries enable the production of antibodies with minimal immunogenicity, reducing the likelihood of adverse immune reactions. These approaches help produce therapeutic antibodies that are less likely to be recognized as foreign by the patient's immune system, thereby reducing the risk of immunogenicity.

Low production yield

How to avoid low yield?

OPTIMIZING CELL CULTURE CONDITIONS

Achieving high yields of functional antibodies is critical, especially for therapeutic applications. Low yields directly contribute to high production costs, as more resources and time are required to produce sufficient quantities of the antibody. This challenge can be addressed by optimizing cell culture conditions, such as nutrient composition, temperature, and pH. Advances in bioreactor technology and the use of high-efficiency expression systems, such as CHO cells, also contribute to increased antibody production, thus helping to reduce costs.

USING EFFICIENT EXPRESSION SYSTEMS

Moreover, advancements in bioreactor technology and the utilization of efficient expression systems like CHO cells play a significant role in boosting antibody production and cutting costs.

PRIORITIZING ANTIBODIES WITH FAVORABLE TRAITS

Another critical aspect is antibody developability, which refers to the ease with which an antibody can be developed into a stable, manufacturable, and effective therapeutic product. Factors influencing developability include the antibody's stability, solubility, and expression levels in production systems. Screening antibodies early in the development process for properties such as high expression levels, stability under manufacturing conditions, and minimal aggregation can enhance developability. By focusing on these characteristics, manufacturers can identify and prioritize antibody candidates that are more likely to yield high quantities and maintain their functional integrity, ultimately reducing production costs and improving the efficiency of the development pipeline.

Post-translational Modifications

How to address post-translational modifications?

Proper post-translational modifications, including glycosylation, are essential for the stability and function of antibodies. Mammalian expression systems are preferred for producing antibodies with human-like glycosylation patterns. However, even in these systems, glycosylation profiles can vary, potentially affecting the efficacy and safety of therapeutic antibodies.

ENGINEERING CELL LINES

Engineering cell lines to produce consistent glycosylation patterns and using glycoengineering techniques can help address this issue.

Immunogenic glycosylation profiles

How to address immune responses?

USING MAMMALIAN CELLS

Non-human glycosylation profiles can trigger immune responses in patients. This challenge is particularly relevant when using non-mammalian expression systems. To overcome this, antibodies are often produced in mammalian cells, which are more likely to perform human-like glycosylation.

USING GLYCOSYLATION PATTERNS

Additionally, glycoengineering techniques can be employed to modify glycosylation patterns and to reduce immunogenicity.

Aggregation and stability

How to achieve stability during the whole process?

OPTIMIZING PRODUCTION CONDITIONS

Antibody aggregation can lead to reduced efficacy and increased immunogenicity. Ensuring the stability of antibodies during production, purification, and storage is crucial. This can be achieved by optimizing production conditions, using stabilizing agents, and employing protein engineering techniques to enhance the inherent stability of antibodies.

USING ANALYTICAL METHODS

Advanced analytical methods are also used to monitor and control aggregation throughout the production process.

Integration of artificial intelligence in antibody development

Artificial intelligence (AI) is emerging as a game-changer across the entire life cycle of therapeutic and diagnostic antibodies—from antigen design to bioproduction. By accelerating critical phases and improving decision-making, AI helps save time, reduce costs, and improve the likelihood of success.

ANTIGEN DESIGN

One of the first areas where AI adds tangible value is antigen design. This is particularly true for complex targets such as membrane proteins, where producing the full-length protein in nanodiscs or overexpressing it in cells can be costly and time-consuming. AI-driven modeling can predict antigenic regions and guide the design of stable and immunogenic antigen constructs, eliminating unnecessary steps and increasing efficiency early on.

AFFINITY MATURATION

Traditionally, antibody affinity maturation involves labor-intensive processes like site-directed mutagenesis, library construction, and phage display screening. Today, AI can help bypass some of these steps by generating optimized antibody variants in silico. These candidates can then be validated experimentally. This hybrid approach significantly shortens development timelines—a major advantage in therapeutic antibody discovery, where speed can be crucial.

REDUCING IMMUNOGENICITY

AI also facilitates antibody humanization, a critical step to reduce immunogenicity in clinical applications. Based on the structure or sequence of the parental antibody, AI algorithms can identify key binding residues and graft them onto a human framework. By simultaneously predicting properties such as binding affinity and immunogenicity, AI can propose the most promising humanized candidates for lab testing.

DEVELOPABILITY OPTIMIZATION

Therapeutic antibodies must not only bind effectively but also be manufacturable and stable. Developability assessments—evaluating solubility, thermal stability, and expression yields—can be enhanced with AI. Algorithms can detect liabilities such as free cysteines or oxidation-prone residues and propose corrective mutations. This proactive approach helps derisk downstream processes, often in just minutes.

ADDITIONAL APPLICATIONS OF AI IN ANTIBODY DEVELOPMENT

Beyond the main development stages, AI is being explored or already applied in other critical areas:

- **Epitope prediction** to inform rational immunogen design
- **Paratope mapping** for structural antibody engineering
- **In silico screening of antibody libraries** to prioritize high-affinity candidates
- **Predictive immunogenicity assessment** based on T-cell epitope mapping
- **De novo antibody generation,** enabling rapid exploration of sequence space for novel binders
- **Automation of sequence clustering and lineage analysis,** especially for single B-cell and NGS datasets

These developments demonstrate that AI is not a simple add-on—it is becoming an integral part of modern antibody discovery pipelines.

At ProteoGenix, we see AI not as an end goal, but as a powerful enabler—when combined with deep scientific expertise, advanced bioinformatics, and in-house experimental validation. Our approach is built on four complementary pillars: scientific knowledge, data science, machine learning, and laboratory testing. This integration ensures our AI tools act as intelligent assistants, not black boxes, with experts guiding every step—from data curation to model refinement. Tailored specifically for therapeutic and diagnostic antibody development, our AI enhances key stages such as antigen design, epitope mapping, affinity maturation, rapid humanization, and developability profiling—and soon, de novo antibody generation. The result: smarter designs, faster timelines, and greater confidence in your antibody candidates.

Perspectives and future trends in antibody production

Advancements in Synthetic Biology | Novel Expression Systems

Advancements in Synthetic Biology

Synthetic biology offers new possibilities for the production of monoclonal antibodies. Techniques such as CRISPR-Cas9 gene editing allow precise modifications to be made to cell lines, enhancing their productivity and ensuring consistent post-translational modifications. Synthetic biology also enables the design of entirely new antibody formats, such as bispecific antibodies and antibody-drug conjugates (ADCs), which can target multiple antigens or deliver therapeutic payloads directly to disease sites.

Engineered microbes and yeast are being developed as alternative expression systems that can produce antibodies with human-like glycosylation patterns. These systems promise to reduce production costs and increase scalability, making antibody therapies more accessible.

Novel Expression Systems

The development of novel expression systems is another exciting trend in antibody production. Mammalian cells, particularly CHO cells, remain the gold standard, but new systems are being explored to overcome their limitations. For example, plant-based expression systems offer a cost- effective and scalable alternative, with the added benefit of reduced risk of contamination by human pathogens.

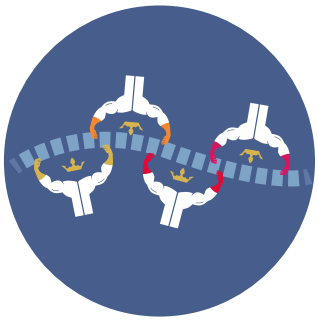
Cell-free expression systems are also gaining attention. These systems use extracts from cells to produce proteins in vitro, allowing for rapid and flexible production of antibodies. Cell-free systems can be easily scaled and adapted, providing a valuable tool for high-throughput screening.

Antibody production at ProteoGenix

With more than 25 years of experience and a track record of over 6,000 successful projects, our team of experts possesses the necessary knowledge and skills to handle even the most challenging custom antibody projects. Whether you are working on innovative therapeutics or pioneering research, our tailored solutions and exceptional quality assurance will support you in reaching your objectives.

Our antibody production services

MONOCLONAL ANTIBODY PRODUCTION



POLYCLONAL ANTIBODY PRODUCTION

At ProteoGenix, we are committed to supporting you through every stage of your custom antibody project, from generation to large-scale production. With our unique approach that combines advanced wet lab techniques with cutting-edge AI platforms, we guide you through the entire antibody production process. We provide a comprehensive suite of services, including developability assessment and improvement, selection of the best expression system for your needs, and optimization of expression conditions.

Why choose ProteoGenix's antibody production services?

Dedicated PhD account managers

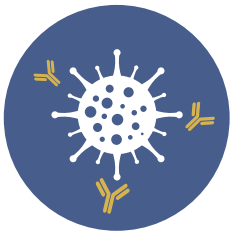
We put at your disposal highly skilled account managers who guide you along your decision-making process.

Tailor-made solutions

Each project requires a specific approach to get the best results. We adapt our services to your goals.

Market's best guarantees

Get access to market's best guarantees (yield, reactivity, etc.).



Antigen design and production

Our experienced team will help you design the best antigen synthesis strategy for superior antibody production results.

Antibody format & species diversity

Produce your antibodies in any species (including human) and format (scFv, Fab, VHH, full-length, or antibody-fusion proteins).

An impactful story

TELUM Therapeutics Partners with ProteoGenix for Custom Monoclonal Antibody Services in EpIeTTX1 Pharmacokinetic Monitoring

How ProteoGenix's Custom Monoclonal Antibody Services Delivered a Breakthrough for TELUM Therapeutics Pharmacokinetic Monitoring

In the fight against multidrug-resistant bacterial infections, TELUM Therapeutics is pioneering precision-engineered therapies by thinking outside of the drug design box. Their lead therapeutic candidate, EpIeTTX1, targets a deadly drug-resistant human pathogen, Acinetobacter baumannii, which recently made the World Health Organization Bacterial Priority Pathogen List. Advancing this innovative protein-based therapeutic required understanding how quickly this bacteria-killing protein is metabolized once it enters the body (pharmacokinetics).

Such a task required a highly specific monoclonal antibody that recognized the full-length, EpIeTTX1 protein without cross-reacting to EpIeTTX1 protein fragments produced once inside the body.

However, there was a problem. That monoclonal antibody didn't exist.

Dr. Robert Diez, TELUM Therapeutics Chief Operating Officer (COO) explains, "We initially considered internal development of the antibody, but quickly recognized the technical complexity and resource intensity of this task. [W]e had engaged with other vendors, but none provided the technical insight, flexibility, and collaborative spirit that ProteoGenix demonstrated."

Dr. Diez reveals what game-changing approaches ProteoGenix used to turn their dream antibody into a reality in this exclusive interview.

ABOUT TELUM THERAPEUTICS: COMBATING ANTIBIOTIC RESISTANCE

Founded in 2019 and headquartered in Noáin, Navarra, Spain, TELUM Therapeutics is a biotechnology company dedicated to tackling multidrug-resistant bacterial infections. "By leveraging synthetic biology and AI-driven machine learning, we aim to develop precision-engineered therapies," says Dr. Diez. Their mission is to deliver novel antimicrobial agents, with EpIeTTX1 leading the charge against Acinetobacter baumannii, a critical threat in healthcare settings.

The EpIeTTX1 project focuses on a therapeutic protein designed to harness the bactericidal power of engineered phage lytic enzymes. However, developing the therapy requires understanding how EpIeTTX1 is absorbed, distributed, metabolized, and eliminated in the body. "[Pharmacokinetic] data ensures optimal dosing, maximizes efficacy, and minimizes side effects," Dr. Diez explains, underscoring its necessity in advancing EpIeTTX1 drug development toward clinical trials.

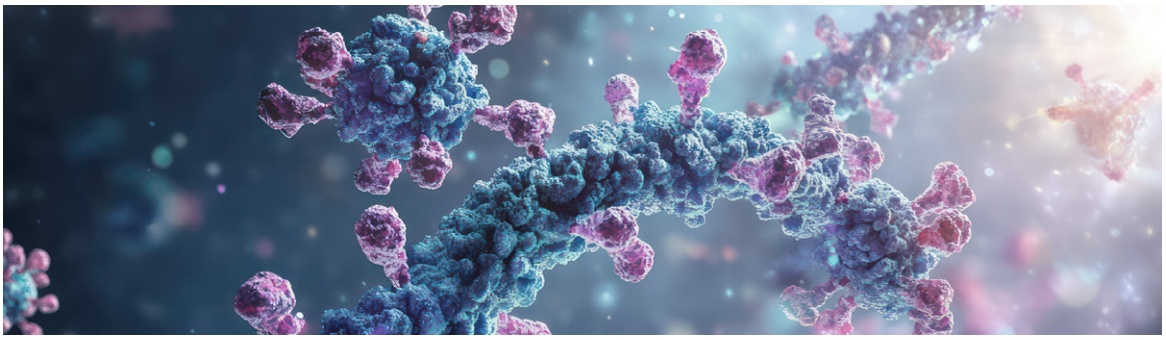
The Challenge: Detecting Intact EpIeTTX1 with Precision

Developing antibodies for EpIeTTX1 was no small feat. TELUM needed a custom monoclonal antibody that could exclusively recognize the intact protein in blood plasma, avoiding cross-reactivity with degraded fragments. “The structural similarity between the intact protein and its degradation products made this exceptionally challenging,” Dr. Diez notes. “Any cross-reactivity could lead to erroneous [pharmacokinetic] data, affecting dosing and therapeutic assessment.”

TELUM initially considered in-house antibody development but recognized the task’s complexity. Previous engagements with other vendors fell short, lacking the technical insight and flexibility required. “We needed a partner who could deliver not just antibodies, but highly specific analytical assays,” Dr. Diez recalls. This set the stage for ProteoGenix to step in and address a critical roadblock.

> ***“The specificity required was a high bar—ProteoGenix cleared it with expertise and precision.”***

Dr. Robert Diez, COO, TELUM Therapeutics



Why TELUM Chose ProteoGenix's Custom Monoclonal Antibody Services.

When selecting a partner, TELUM evaluated several providers, but ProteoGenix stood out. "Their track record with complex, custom antibody programs and their ability to align with our scientific and regulatory demands made them the clear choice," says Dr. Diez. Unlike vendors offering generic workflows, ProteoGenix offered tailored solutions for EpleTTX1's unique needs.

Key qualities that set ProteoGenix apart included:

- **Technical Expertise:** Deep knowledge in hybridoma development, epitope targeting, and assay design, including proteolytic degradation models
- **Flexibility:** Customized immunization and screening protocols to fit TELUM's budget and timeline as an early-stage biotech.
- **Collaboration:** "Their project manager maintained proactive communication…" Dr. Diez emphasizes.

This partnership approach ensured ProteoGenix wasn't just a vendor but a strategic ally in TELUM's mission.

ProteoGenix's Approach: Precision and Innovation

To meet TELUM's challenge, ProteoGenix deployed a sophisticated, multi-step strategy. "Developing antibodies that only recognized the intact EpleTTX1 required extreme specificity to conformational epitopes," Dr. Diez explains. ProteoGenix's hybridoma technology was ideal, offering high specificity and scalable production.

A stand out innovation was their custom proteolytic degradation assay, which simulated EpleTTX1's breakdown in plasma. "This required deep molecular biology expertise—protease selection, protein engineering, and analytical method development," Dr. Diez notes. The assay tested antibody candidates under real-world conditions, ensuring only those binding the intact protein advanced.

ProteoGenix's validation process was equally rigorous:

- **Comparative ELISAs** eliminated cross-reactive candidates
- **Western blotting and epitope mapping** confirmed binding to the native protein.
- **In vitro degradation assays** ensured robustness in complex biological matrices like blood.

Their flexibility also shone through. "They adapted protocols to fit our budget without compromising quality. ProteoGenix's degradation assay was a game-changer, giving us confidence in the antibodies' specificity." Dr. Diez says, highlighting adjustments to immunization and antibody screening pipelines. This, along with the regular GO/No-GO checkpoints and proactive communication, kept TELUM Therapeutics confident and informed.

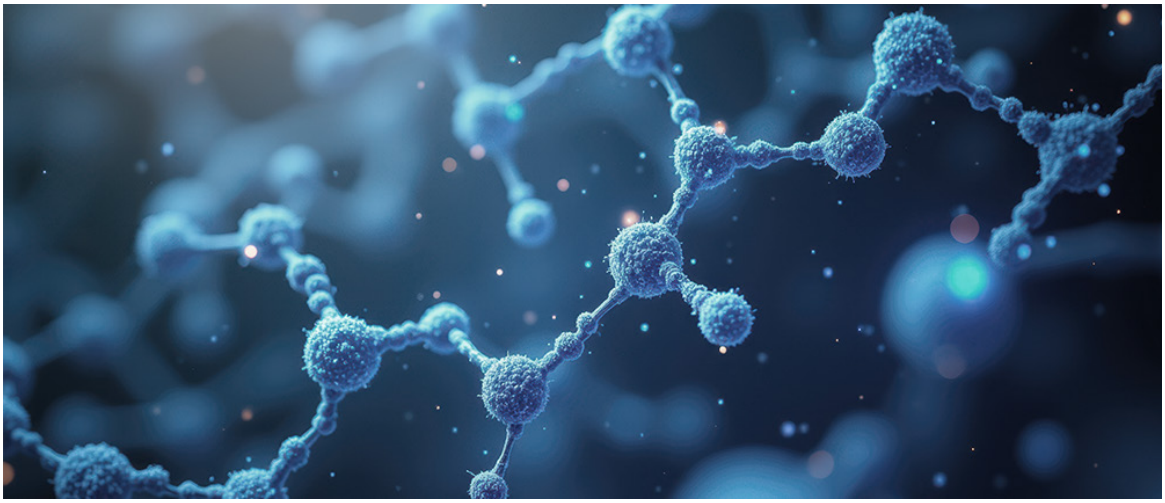
THE JOURNEY: KEY MILESTONES

The project unfolded through critical milestones, each advancing TELUM's goal of robust pharmacokinetic monitoring:

- **Antigen Design and Immunization:** ProteoGenix optimized an immunogen to target EpIeTTX1's conformational epitopes, eliciting a strong immune response.
- **Degradation Protocol Development:** A custom assay mimicked plasma breakdown, enabling precise candidate selection.
- **Hybridoma Screening:** Hundreds of clones were screened via ELISA and orthogonal assays, narrowing to 94 positive hits.
- **Top 10 Clone Selection:** Ten clones were chosen for their high specificity and minimal cross-reactivity, validated through Western blotting.
- **Extended Applications:** The antibodies supported anti-drug antibody (ADA) assay development, enhancing preclinical studies.

> ***"The collaboration was seamless and scientific, ProteoGenix's team was deeply involved, offering strategic advice at every step.Their responsiveness ensured the project stayed on track, even when technical hurdles arose."***

Dr. Robert Diez, COO, TELUM Therapeutics



THE RESULTS: A BREAKTHROUGH FOR TELUM

ProteoGenix delivered 10 monoclonal antibodies with exceptional sensitivity, detecting EpIeTTX1 in the low nanogram per milliliter (ng/mL) range. These antibodies enabled TELUM to develop a sandwich ELISA assay with:

- **High Specificity:** No signal from degraded fragments, ensuring accurate pharmacokinetic data.
- **Robust Performance:** Excellent linearity and reproducibility across a broad dynamic range.
- **Rapid Implementation:** Quick transition from development to routine use.

"The quality of the antibodies transformed our [pharmacokinetic] studies," Dr. Diez says. TELUM could now:

- Quantify intact EpIeTTX1 in plasma with confidence.
- Determine half-life, clearance, and exposure for dose optimization.
- Lay groundwork for ADA detection and tissue distribution studies.

This milestone "brought us closer to delivering a new therapy for multidrug-resistant infections," explains Dr. Diez. "It validated our R&D platform and provided a complete analytical package for regulatory submissions and clinical trials," Dr. Diez explains. The antibodies also supported new intellectual property and planned publications, strengthening TELUM's position in antimicrobial innovation.

WOULD DR. ROBERT DIEZ RECOMMEND PROTEOGENIX TO HIS PIERS?

Reflecting on the collaboration, Dr. Diez praises ProteoGenix's impact. "The most valuable aspect was their ability to combine technical expertise with collaborative agility," he says. "They understood our challenges, adapted protocols, and provided insights that overcame roadblocks."

The partnership has lasting value:

- **Product Development:** The antibodies are now core to EpIeTTX1's pharmacokinetic and immunogenicity studies.
- **IP and Publications:** New patents and peer-reviewed papers are in progress.
- **Future Collaboration:** TELUM Therapeutics is discussing next-generation antibody projects and diagnostic tools with ProteoGenix.

"I'd recommend ProteoGenix without hesitation," Dr. Diez concludes. "They bring scientific depth, flexibility, and exceptional communication, delivering quality with precision."

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