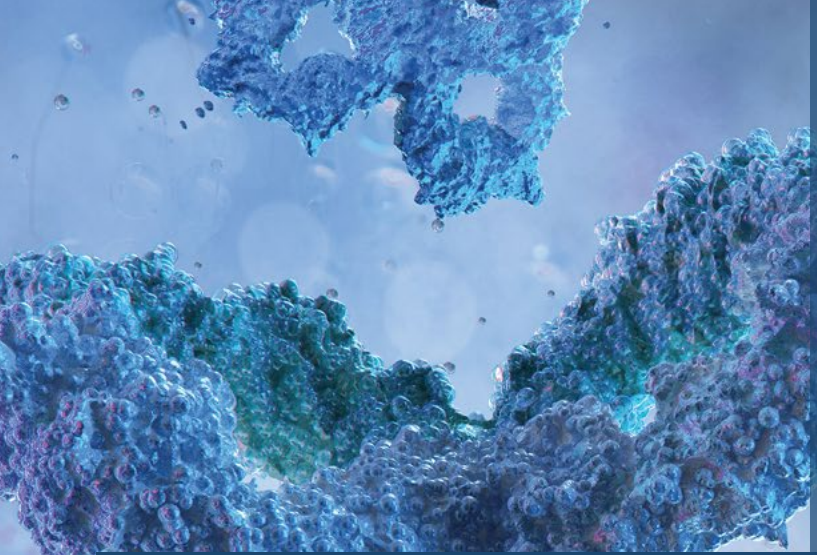


ROCKLAND

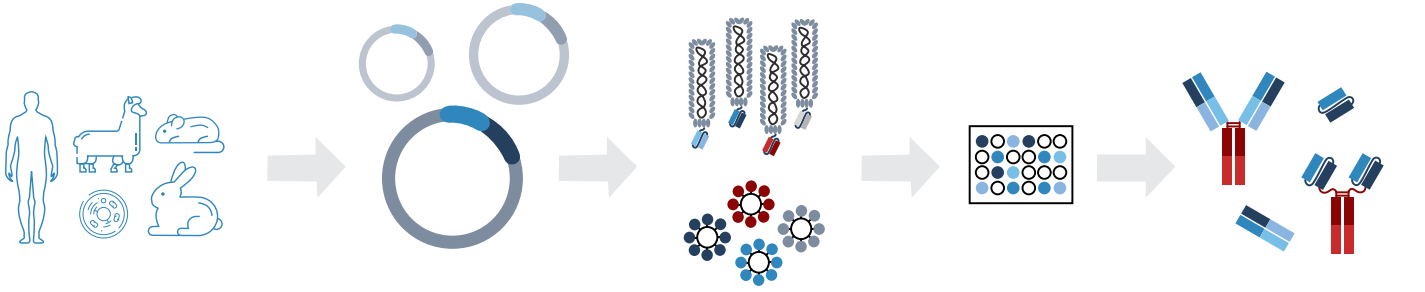
Recombinant Antibody Services

From Rockland

Recombinant antibodies have many advantages over purified natural and traditional hybridoma antibodies. These include supply chain security and improved quality control in production. Rockland can convert your antibody to a recombinant antibody format whether your antibody is sourced from an existing hybridoma cell line, is already a purified antibody protein, or is generated from an antibody display library.

Library Generation & Screening

Libraries can be made from hosts that include mouse, rabbit, alpaca or llama, and human. Construction of the library is generated with antibody genes derived from sources that may include blood (i.e., PBMCs), spleen, bone marrow, or other tissues as appropriate for your project. The library is then screened for the binding specificity of interest. This is typically a biotinylated form of the target, but other screening methodologies can also be used such as panning on solid surfaces (e.g., coated plates) or on cells and even tissues. Selected antibody clones are sequenced, then the gene is synthesized in the format of interest, and the purified recombinant antibody proteins produced for further use.



Donor host (naive or immunized) or cell

Cloned antibody genes

Phage & yeast library construction

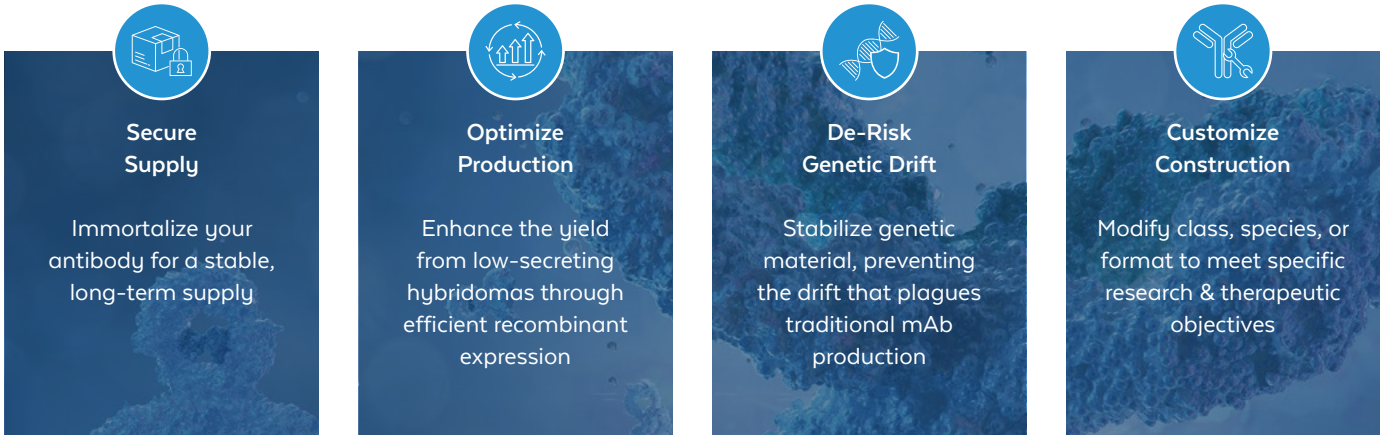
Identified binding clones

Reformatted antibody expression

Hybridoma Conversion to Recombinant

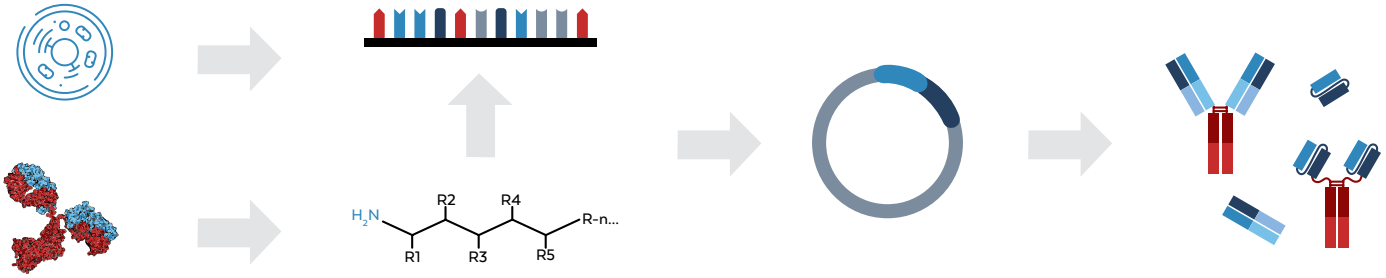
rAbs can be produced in a wide range of formats that include common antibody constant regions (e.g., IgG1 Fc, IgG2 Fc, or others) as well as engineered molecular modifications such as fragments of the antibody (e.g., scFv) and inclusion of molecular tags (e.g., FLAG, poly-His, and a range of other options). Various other options include multi-valency and multi-specificity. We can also functionalize your rAbs via genetic fusions to other proteins or biochemically modify them with enzymes (e.g., horse radish peroxidase, alkaline phosphatase, and others), dyes (e.g., FITC and other fluorescent or chemiluminescent molecules) or other tags and molecules (e.g., biotin, PEG).

Whether your antibody is derived from a hybridoma cell line, a yeast or phage display library, or a de novo protein sequencing effort from a purified antibody preparation, we can improve the production and performance of your antibody by conversion to a rAb format.



Targeting Genetic Drift

One of the most significant challenges in the use of traditional monoclonal antibodies is genetic drift—a random mutation process that occurs during continued cell divisions in hybridoma cell cultures. These mutations can modify the antibody's binding sites, leading to decreased affinity and specificity over time. By transitioning to a recombinant platform, the genetic sequence of your antibody is cloned into a stable expression system, ensuring the preservation of its original properties indefinitely.



Obtain the genetic or protein sequence

Determine gene sequence for cloning

Clone into expression vector

Recombinant antibody expression & purification (up to 1g/L)

Bio-layer Interferometry Services

The label-free Bio-Layer Interferometry (BLI) technology enables the direct detection of specific proteins and other biomolecules in complex mixtures like crude cell culture supernatants and lysates. BLI uses optical-based biosensors to convert biological binding reactions into signals without the use of a detection label for real-time monitoring of changes, while eliminating the need to manipulate individual assay components.

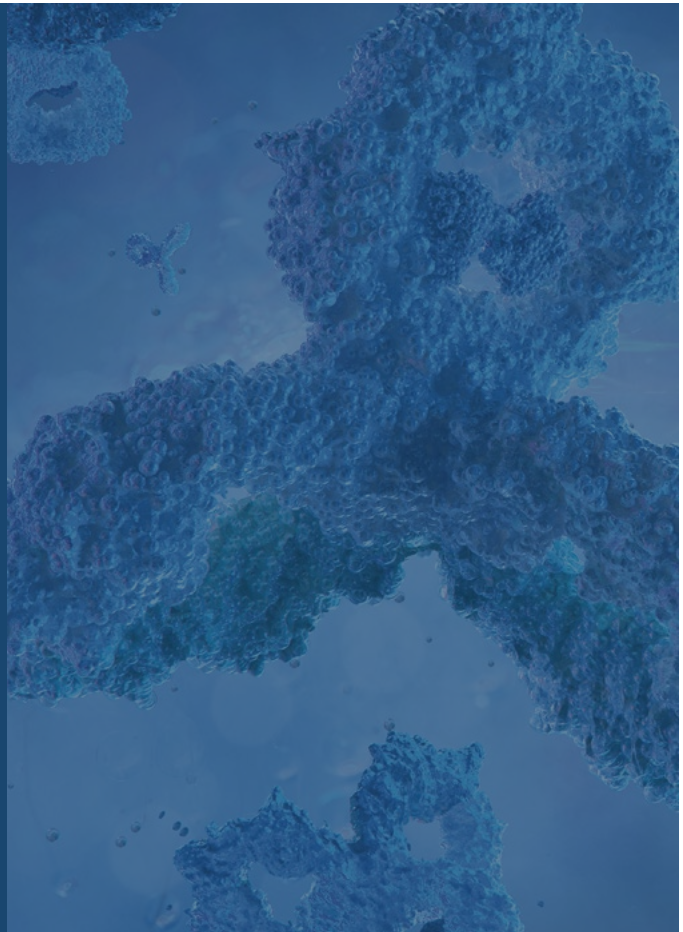
+ Antibody-antigen binding

+ Epitope binning and antibody pairing

+ Quantitation of antibodies or antigens

+ Affinity assessments with kinetic analyses

+ Early-stage screening of hybridomas for rapid & efficient selections



Antibody Affinity Maturation

Affinity maturation can be used to enhance the binding characteristics of your antibody via various affinity maturation approaches. Depending on the present affinity of your antibody, binding affinity can be often improved by at least 10x. For some applications, a weaker binding affinity may be desired and this can be achieved as well, when needed.

Methods used for the generation of sub-libraries, which include error-prone PCR for the introduction of sequence substitutions or the total synthesis and randomization of CDRs in your antibody, are implemented to seek clones with altered binding characteristics and kinetics such as on- and off-rates.

Recombinant VHH Antibodies

We offer an all-inclusive recombinant VHH antibody production service that generates antibodies suitable for a wide range of applications, including cryptic epitope detection, hapten detection, enzyme active site binding, diagnostic imaging, virus neutralization and more.

Our recombinant VHH antibodies are produced using hyperimmunized camelids and single-cell selection from clonal display, providing the desired sensitivity, specificity, and affinity and enabling production in bacterial or mammalian expression systems.

Antigen Preparation
Prior to Month 1

We can use a range of macromolecules as antigens—as long as they can be used under BSL-2 conditions—such as protein, recombinant or purified, synthetic peptide, modified peptide, haptens, whole cell lysate, nucleic acid, lipids, and carbohydrate complexes.

Camelid Immunization
Month 1-2

We generate VHH antibodies in both llama and alpaca, using the immunization schedule you specify and standard or custom adjuvants. We can provide recommendations prior to library construction based on our decades of experience and monitoring of the antibody-mediated immune response (antisera titration).

VHH Library Construction
Month 3

Upon confirmation of a satisfactory immune response, we generate a yeast or phage display library of VHH antibodies. To ensure that the desired binding properties can be found in the library, we analyze critical parameters such as size and diversity.

Advantages of VHH Antibodies

VHH antibodies can be engineered into monomeric or multimeric, multi-specific formats that possess high target specificity, appropriate affinity, and a low tendency to aggregate. They are also less prone to the steric hindrance problems that can challenge conventional antibodies, making recombinant VHH antibodies ideally suited for a range of pre-clinical diagnostic and therapeutic applications.

Biopanning & Clone Isolation
Month 3

To isolate clones that bind to the antigen of interest, we use proprietary affinity selection technologies that we've developed over decades of successful projects.

Antibody Validation
Month 4

Isolated single clones are validated using fit-for-purpose studies that include characterization of specificity (binding to the intended target) and cross-reactivity (binding to unrelated antigens) in the conditions relevant to the applications of interest. We can also perform further custom validation for affinity determination, antibody pair identification, and more.

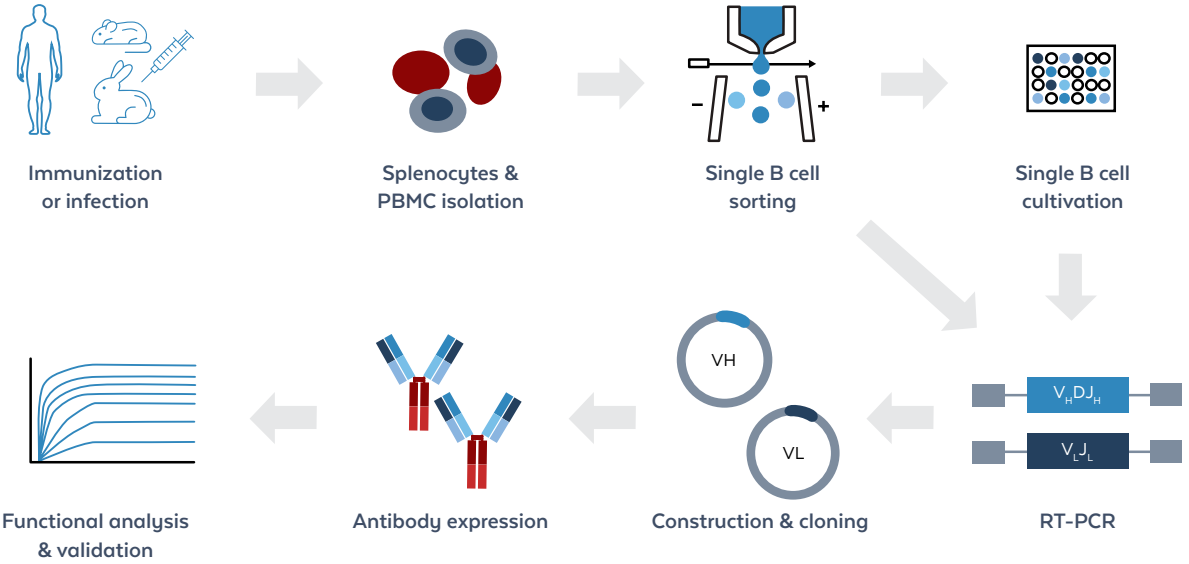
Antibody Production
Month 5

Clones are expressed in a bacterial system at the desired scale and purified at levels suitable for binding and functional studies.

We can also express recombinant VHH antibodies in eukaryotic systems for custom antibody formats and/or applications and can use custom signal sequences or N- or C-terminal tags when required.

Single B Cell Antibody Cloning

Our single B cell antibody cloning services accelerate the identification and development of highly specific monoclonal (recombinant) antibodies. After immunization or exposure of a host species, antigen-specific antibody-producing cells can be sorted by flow cytometry and placed into culture for cell cloning and subsequent molecular cloning of antibody genes for recombinant antibody generation. Molecular cloning, either through construction of antibody display libraries or directly cloning antibody genes from isolated B cells into antibody expression vectors, allows for rapid generation of key antibody specificities with decreased loss of diversity. No matter the sample size or target, Rockland offers an end-to-end solution from immunization through validation to streamline your monoclonal and recombinant antibody discovery and development.



Executing on Challenging Projects

With over 60 years of experience, Rockland has amassed expansive knowledge in all phases of product development. We pride ourselves on being a partner to our clients, offering our expertise to solve the most challenging projects. As a full-service provider, we are able to meet the unique needs of the industry with fully traceable manufacturing and quality assurance systems that allow for a lifetime supply of critical reagents.

End-to-end Solutions

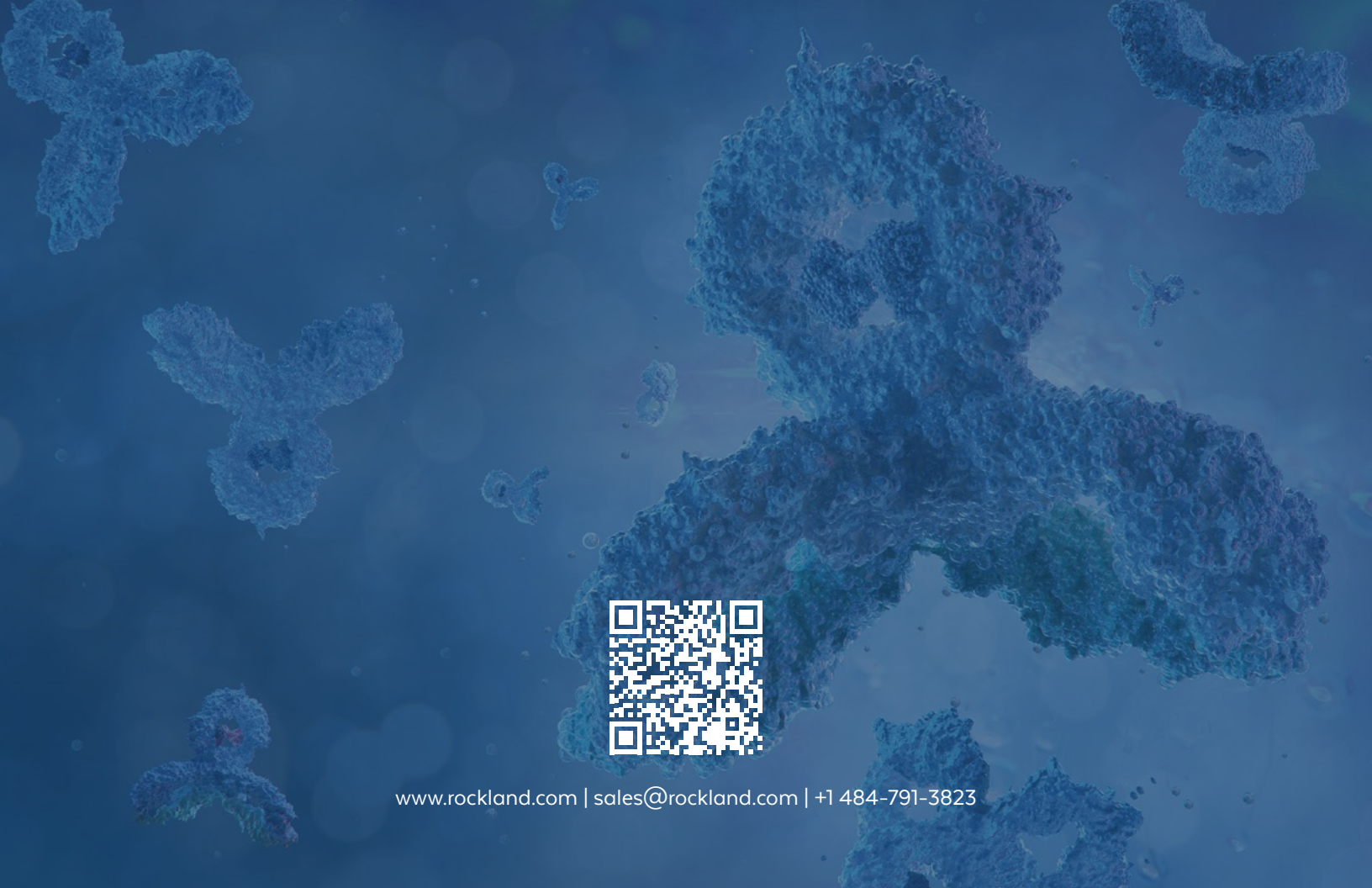
Over a dozen host species with molecular biology & protein expression services, cell culture suites & lysate development

Supply Chain Security

Control of the entire manufacturing process allows for reproducible, qualified, repeat production & long-term supply chain stability

Bulk & Scale-Up Capabilities

Our in-house R&D and quality systems ensure minimal lot-to-lot variation & scalable quantities to >gram quantity scale



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