Single Domain (VHH) Antibody Discovery

Next-Generation Biotherapeutic Applications

At Twist Biopharma Solutions, we leverage *in vitro* and *in vivo* antibody discovery platforms that have been proven to deliver high-affinity, diverse biologics against difficult or cell-surface targets under tight timelines. Our integrated services can produce well-characterized, humanized antibodies across various therapeutic applications:

- Immuno-Oncology and CAR-T targets
- GPCRs
- Type I and Type II cell membrane proteins
- Ion channels
- Small molecule transporters
- Infectious disease targets
- Inflammation targets

Regardless of how you use it, Twist Biopharma Solutions has multiple approaches for your various nanobody applications. Our scientific experts will collaborate with you to determine the best technology or platform to discover your VHHs and deliver high-quality antibodies to meet your unique specifications and goals.

Explore our complementary services to enhance your antibody discovery experience and work with a single provider to deliver the best antibodies for your campaign.

CAPABILITIES

- Humanized VHH libraries ready-to-use or accessible through flexible licensing models
- Alpaca immunizations with Beacon B-cell screening for accelerated discovery timelines
- Custom immune library generation with experience in a variety of antigen types and difficult, cell-based targets
- Complementary services include:
  - *In silico* humanization
  - AI/ML-supported NGS sequencing
  - HT VHH-Fc production
  - Antibody characterization with state-of-the-art technologies
  - Bispecific matrixing reformatting
  - Affinity maturation and optimization
  - Antibody engineering and optimization
  - High-Throughput antibody production and characterization

BENEFITS

- Superior VHH discovery against cell-surface targets
- Ready-to-use VHH libraries for faster hits
- Various approaches for maximum diversity
- Rapid turnaround times with direct screening on IgG2/3-secreting camelid B-cells
- Production of stable and robust antibodies for easy engineering and manufacturing
- Flexible models including royalty- and milestone-free campaigns

Library of Libraries (2-5 months to sequence)

3-5 WEEKS (OPTIONAL)	3-4 WEEKS	2-4 WEEKS	5-8 WEEKS
Antigen and Screening Tool Production	Phage Display Panning: Bread or Cell Based	Screening and Sanger or NGS Sequencing	Recombinant Antibody Expression (+Downstream Engineering/Humanization)

B Cell Screening (2-5 months to sequence)

3-5 WEEKS (OPTIONAL)	12 WEEKS	1 DAY	2 WEEKS	5-8 WEEKS
Antigen and Screening Tool Production	Alpaca Immunization	Beacon-Based B Cell Screening	B Cell Sequencing	Recombinant Antibody Expression (+Downstream Engineering/Humanization)

Immune Libraries (2-5 months to sequence)

3-5 WEEKS (OPTIONAL)	12 WEEKS	2-3 WEEKS	3-4 WEEKS	2-4 WEEKS	5-8 WEEKS
Antigen and Screening Tool Production	Alpaca Immunization	Immune Library Generation	Phage Display Panning: Bead or Cell Based	Screening and Sanger or NGS Sequencing	Recombinant Antibody Expression (+Downstream Engineering/Humanization)

For Research Use Only. Not for use in diagnostic procedures

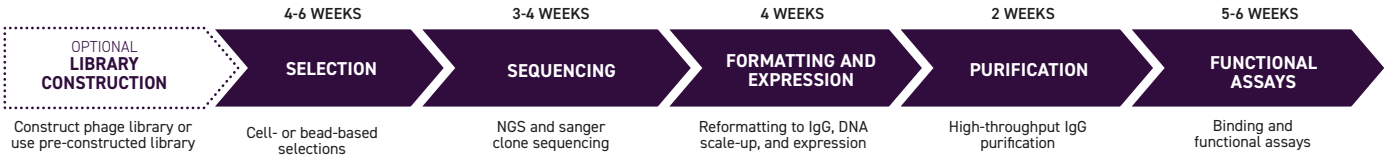
In vitro discovery

Twist Bioscience's DNA writing technology offers significant advantages in creating VHH libraries for antibody discovery. By leveraging high-throughput DNA synthesis, Twist can precisely engineer diverse VHH sequences, allowing for the generation of large, customized libraries with tailored characteristics. This enables researchers to explore a vast, pre-defined sequence space, accelerating the identification of high-affinity VHHs against specific targets and facilitating more efficient antibody discovery and development.

Twist Biopharma Solutions uses multiple novel VHH methods that combine synthetic and natural approaches to maximize diversity, creating high-quality VHH libraries for use against any protein target. These proprietary, naïve single domain libraries are ideal for initiating antibody discovery and development. Their modular nature supports creation of bi- or multi-specific antibodies ideal for developing next-generation therapies. Our VHH Libraries have been validated against challenging targets.

LIBRARY DEVELOPMENT PROCESS

The single-domain antibody library development leverages the advantages of several of Twist's proprietary methods, creating highly diverse phage display libraries in 3-4 weeks. These VHH libraries are part of Twist Biopharma Solutions' "Library of Libraries" and follow a rigorous and specialized process defined by our experienced scientists to produce the highest-quality antibodies.

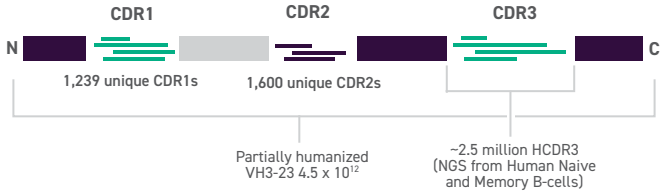


SINGLE DOMAIN ANTIBODY LIBRARIES

VHH hShuffle HI

Unique method shuffles millions of llama and human CDR sequences within the context of a partially humanized VHH framework.

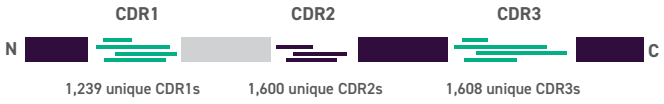
- Natural llama CDR1/2 sequences
- Human CDR3 sequence replaces llama CDR3
- Increases CDR3 diversity from 1,600 to >2 million unique CDR3s
- Partially humanized VH3-23 VHH framework



VHH hShuffle

Unique method shuffles thousands of natural llama CDR sequences within the context of a partially humanized VHH framework.

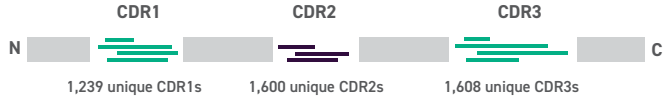
- Each unique CDR is individually synthesized
- Shuffled CDRs for theoretical library diversity of 3.2 x 10^9
- Partially humanized VHH framework
- Framework 1, 3, and 4 humanized using human germline DP-47 framework
- Lowers library immunogenicity for therapeutic development



VHH Shuffle

Unique method shuffles thousands of natural llama CDR sequences within the context of a llama consensus framework.

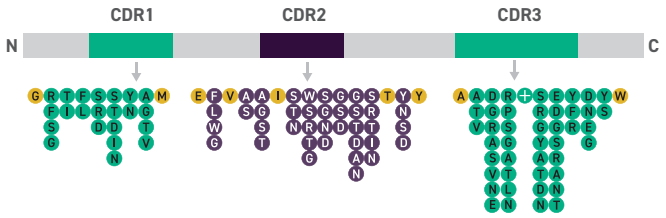
- Each unique CDR is individually synthesized
- Final diversity of library > theoretical diversity of 3.2 x 10^9



VHH Ratio

Synthetic oligo pools model the natural VHH repertoire.

- 2,391 CDR sequences analyzed for position-specific variation
- Controlled CDR diversity introduced into the library
- Amino acid ratios randomized at different positions within CDRs

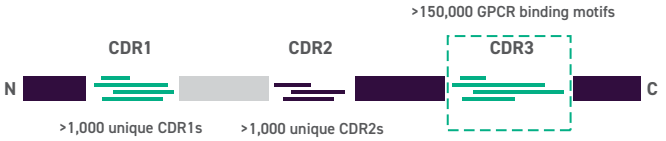


Loop length 0–13 amino acids. All amino acids except Cys and Met.

VHH hShuffle GPCR

High-diversity GPCR motifs in CDR3.

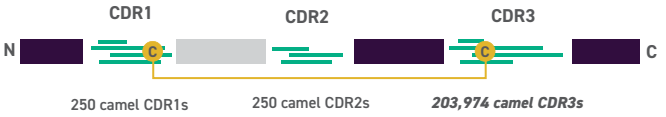
- GPCR binding motifs are taken from GPCR 2.0 library
- These GPCR motifs were incorporated into the VHH hShuffle CDR3
- Final library diversity: 1 x 10^10



hCamel Bactrain (TL38)

Greater HCDR3 diversity derived from naturally evolved VHHs.

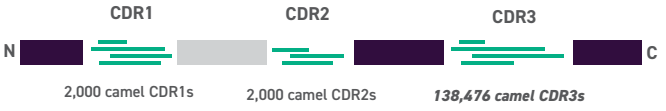
- Disulfide in H1-H3 for enhanced stability
- 2 Cys are like the 2 humps in Bactrian camels



hCamel Zero (TL84)

Greater HCDR3 diversity derived from naturally evolved VHHs.

- Engineered to have zero Cys in CDRs
- Additional diversity in CDR1 and CDR2



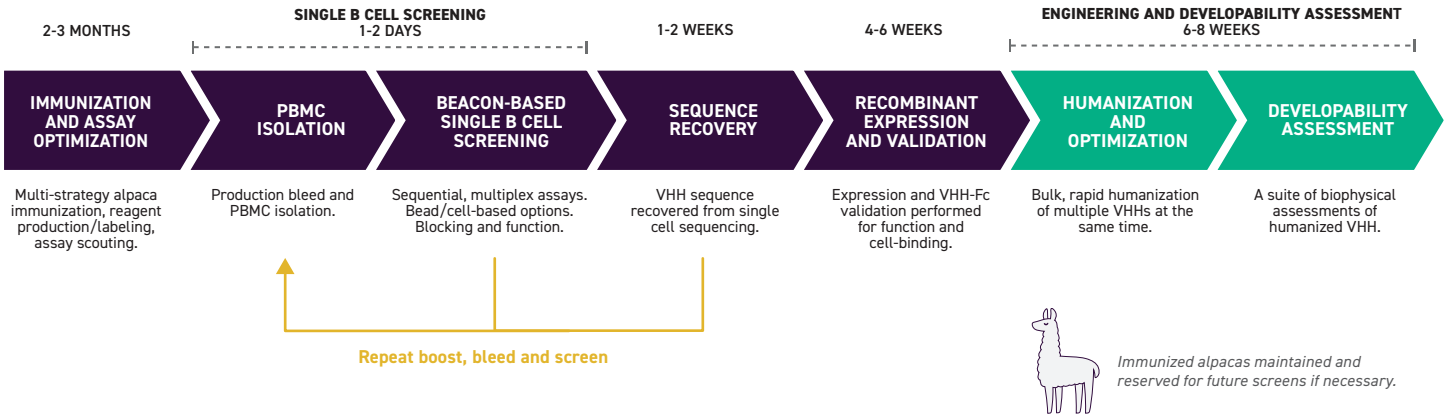
In vivo discovery

Using Beacon® B-cell screening for discovering VHH antibodies offers a unique advantage by directly interrogating the immune repertoire of camelids. This *in vivo* approach allows for the identification of VHH antibodies with desired specificities and functional properties and harnesses the natural diversity and affinity maturation processes of the immune system. This approach potentially yields antibodies with superior binding affinities and biological activities for various applications, including research tools and therapeutics development.

SINGLE B-CELL SCREENING FOR SINGLE-DOMAIN ANTIBODIES

Twist Biopharma Solutions has successfully delivered purified, recombinantly expressed VHHs with affinities in single-digit ranges from discovery campaigns leveraging the combination of alpaca immunization and direct screening of IgG2/3 secreting B-cells. Our experienced team of scientists has optimized the Beacon platform to use optofluidic technology to precisely manipulate the entrapment of single B-cells into individual nanopens within a microfluidic chip. The assays are then performed on-chip using fluorescence imaging and validated off-Beacon for binding kinetics to deliver you the most promising hits.

VHH Discovery for Therapeutics Development



BENEFITS OF TWIST'S APPROACH

Superior VHH discovery against cell surface targets

- Upfront on-cell screening
- Leverages inherent natural diversity

High-affinity antibodies

- Natural *in vivo* affinity maturation
- Beacon-based screening

Rapid timelines

Direct screening on IgG2/3 secreting camelid B-cells

Royalty free

No royalty and milestone requirements initiated in 2024

Best of both worlds: immune libraries for VHH discovery

At Twist Biopharma Solutions, our scientists collaborate with you on a custom, hybrid *in vitro/in vivo* approach to VHH antibody discovery. This process includes first immunizing camelids with the target antigen and then isolating peripheral blood lymphocytes from the immunized animals. Our expertise comes in designing immunization protocols and selecting appropriate adjuvants to generate a diverse immune response. The isolated lymphocytes are then used to construct a library of VHH antibody sequences, typically by cloning the variable domains into expression vectors. Our experienced scientists then deliver you a custom library with high diversity and desirable biophysical profiles to ensure specificity and affinity.

Alpaca Immunization (TIMELINE 12-14 WEEKS)

Small but mighty

VHH antibodies, also known as nanobodies or single domain antibodies (sdAb), are small antibody fragments derived from camelids that offer several advantages in biotherapeutic discovery and developing bispecific or multi-specific antibodies. Their small size allows for more effective tissue penetration and access to cryptic epitopes. Additionally, their high stability and solubility simplify manufacturing processes and improve shelf life. Some of their applications include:

THERAPEUTICS

Therapeutics in the areas of oncology, autoimmune, and infectious disease. Nanobodies can selectively bind to to epitopes usually sterically hindered by traditional antibodies. This makes them strong candidates for biotherapeutics that can block signaling pathways involved in tumor development, be small enough for efficient tumor penetration and rapid clearance, or effectively cross the blood-brain barrier.

DIAGNOSTICS

Diagnostics at point-of-care or as tools in medical imaging. Single-domain antibodies can be engineered to detect pathogens, toxins, or biomarkers, which enables easy integration into portable diagnostic platforms. Additionally, their small size and rapid tissue penetration and clearance make fluorescently labeled nanobodies attractive platforms for in vivo imaging.

RESEARCH

Critical reagents or research tools with high specificity and affinity. Nanobodies can be engineered to bind to difficult target proteins. Coupled with their small size, these single-domain antibodies are valuable tools for protein detection, analysis, and stabilizing reagents.

Antibody discovery with a Twist

The dynamic process in discovering and developing antibodies relies on the iterative design-make-test-analyze cycle. This systematic approach involves generating a diverse library of antibodies, followed by thorough testing to identify those with the desired binding specificity and functionality. Benefits to your antibody discovery campaign include:

- Rapid and flexible response for real-time adjustments and optimizations
- Speed and agility that puts you on the critical path to the clinic
- Reproducibility and reliability in the generated antibodies
- Centralized data management to ensure precision and quality
- More effective and targeted therapeutic candidates

Complementary services to optimize your VHH antibodies

ANTIBODY ENGINEERING AND OPTIMIZATION

Affinity Maturation
Rapid, high-diversity in silico library screening to deliver you antibody variants with improved affinity profile

Humanization
An add-on or stand-alone service to engineer fully human antibodies while maintaining desired affinity, safety, and efficacy profiles

ANTIBODY CHARACTERIZATION

Binding confirmation
ELISA, Flow Cytometry, Octet

Affinity ranking and KD determination
SPR, Octet, Flow Cytometry

Epitope binning
SPR, Octet

Functional and bioanalytical
Internalization, Cytotoxicity, Ligand Blocking, Endotoxin Testing, Recruitment or Inhibition, Cross-Reactivity

Developability assessment
In silico Sequence Analysis, CIC, AC-SINS, HIC, BVP-ELISA, aSEC, Thermo stability (NanoDSF/DLS)

EXPRESS ANTIBODY PRODUCTION AND SEQUENCING

Small- to Medium-Scale HT VHH-Fc Production
- CHO (13-18 days)
- HEK (10-15 days)

Bispecific Matrixing
Reformatting scFv, Fab, bi- and multi-specifics to design and generate complex molecular formats

NGS Sequencing
AI/ML Support for enhanced lead selection

What can we help you discover?

Twist Biopharma Solutions is committed to helping you realize the potential of your biggest biologic development programs. Wherever you are in the development pipeline we have a solution that will elevate you to the next level of discovery

LEARN MORE

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