



*A Greater Area Bay company dedicated to
Developing First-In-Class Single Domain Antibody-
Based Products*

About Jotbody

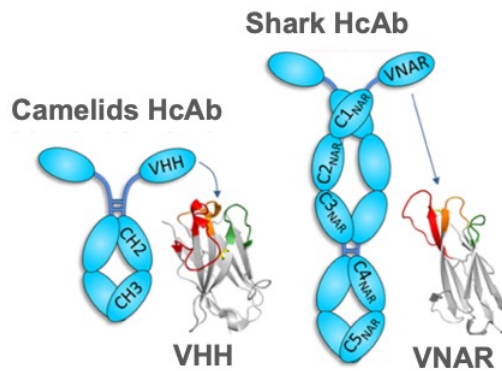
Jotbody (HK) Limited is a biotechnology company with dual sites operating in Hong Kong and Shenzhen (China). Jotbody is on the cutting edge of single-domain antibody discovery, production, and application. Jotbody is dedicated to offering single-domain antibody discovery CRO services and single-domain antibody-based research reagents of outstanding quality to the global scientific community.

 jotbody@jotbody.net

 www.jotbody.net

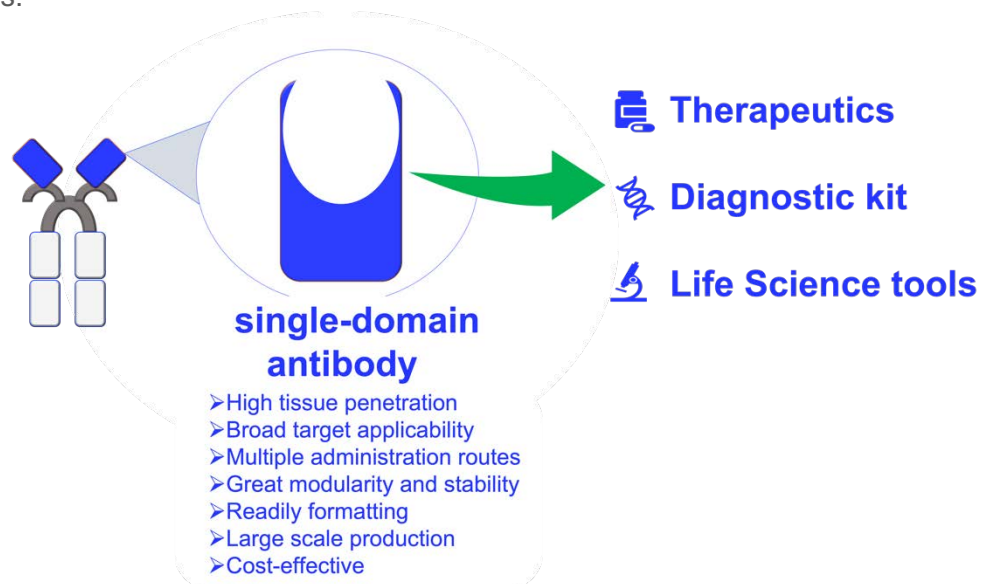
Single domain antibody

Single domain antibody (sdAb), also called nanobodies, is the variable region isolated from the antigen-binding variable domain (VHH) of camel heavy-chain only antibody or the variable domain (vNAR) of cartilaginous fish immunoglobulin new antigen receptor (IgNAR). With a size of just 15 kDa (2~4 nm), sdAb is the smallest antigen-binding fragment today discovered.



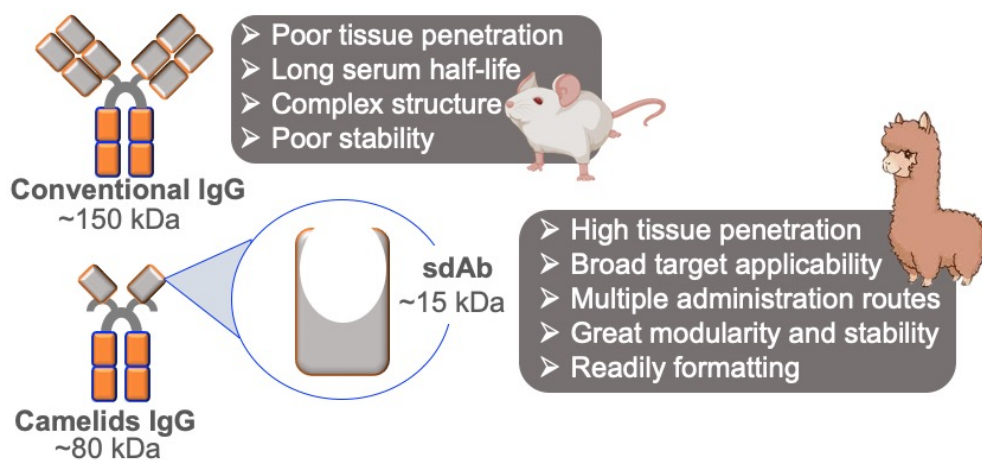
Single domain antibody: a new player in antibody-based therapy

Single domain antibody (sdAb), the smallest known antigen-binding fragment, is fated to leave an indelible mark on clinical medicine, diagnostic and basic research. Indeed, given its unique biological features and remarkable therapeutic versatility, sdAb struck the attention of the entire scientific community and investors, generating a rapid progression of numerous innovative sdAb-based therapeutic platforms.



SdAb vs conventional antibody

sdAb owns a simple architecture, high thermal and chemical stability, good solubility, high tissue penetration, easy modularity, and offers the possibility for large-scale production. SdAbs are becoming a key component in a variety of areas of research, such as medical diagnosis, therapy, precision medicine and basic research.



Characteristics	Single domain antibody	Conventional antibody
Size	~15 kDa	~150 kDa
Binding affinity	pM to nM ranged kD	nM to μ M ranged kD
Batch to Batch consistency	Very high	High (subjected to genetic drift)
Novel epitope	Yes	N/A
Solubility (aggregation escape)	High	Very poor
Stability (temperature and pH)	High	Low

What we offer	
SdAb discovery services: <ul style="list-style-type: none"> Immunized camelids sdAb phage library Naïve camelids sdAb phage library Immunized shark sdAb phage library 	SdAb-based research reagents: <ul style="list-style-type: none"> Primary antibody Secondary antibody Conjugated/unconjugated antibody

We offer high quality products, excellent customer service & technical support:

- Year-round immunization
- Customizable project
- Stress-free animals with clean immune background
- Immunized and naïve libraries
- Multiple host animals available for immunization (camel, alpaca, llama, shark) & vectors (bacterial, mammalian and insect cells)
- Quality assurance (qualified antigen quality, adequate immune titer, mega library)
- Short-cycle workflow
- Proven scientific know-how
- Reliable lab report with timely update
- Unbeatable prices & 100% success rate

Host animals

Our first commitment is to ensure that our animals are properly nourished. Our staff and collaborators are dedicated to providing a comfortable and well-equipped environment that ensures the highest welfare and ethical standards for animal care. Scientists and staff at Jotbody are highly experienced in the immunization of a variety of animals. Their knowledge is indispensable for generating high-quality sdAb since the high titer in the animal blood is the first step to guarantee the success of sdAb isolation.



Alpaca, llama, camel and shark are the host animals that Jotbody offers to our customer to discover sdAb against the intended target.

Our large facilities are able to conduct animal immunization year-round.

Australian livestock

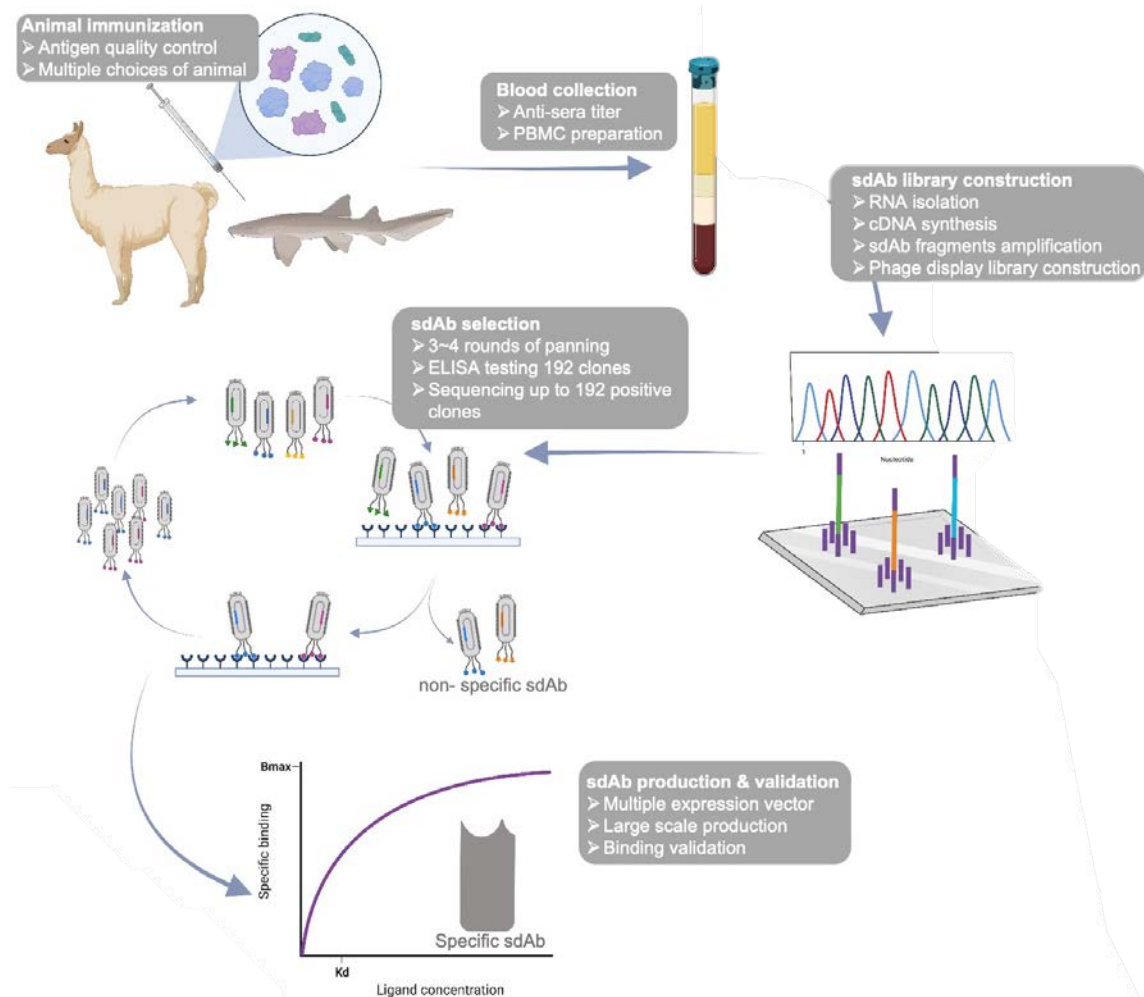
Australia is recognized globally for having the best standards in terms of animal health and quarantine regulation, which is paramount for producing premium quality biological products.



We select and collaborate only with the finest Australian farms that adhere to strict animal farming standards. All of our animals are ethically farmed and have clean immune backgrounds. This ensures the final sdAB products our customers receive are of the highest quality, without a hefty price tag.

Immunized single domain antibody discovery workflow and timeline

Jotbody adopts a cost-effective, customizable, and short-cycle workflow for the discovery and production of sdAb from camel, alpaca, llama, and shark. Through our comprehensive technology platform and service portfolios, our team is best at discovering unique sdAb to meet your specific requirement.



Standard camelids immunization schedule

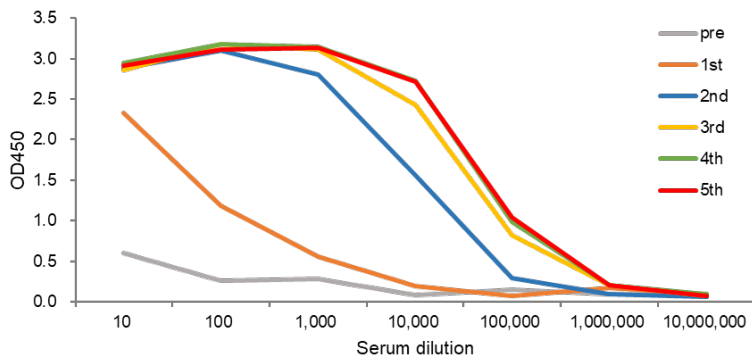
Day	Action	Procedure	Antibody titer test
1	1 st immunization	Pre-immune peripheral blood and serum collection	Pre-immune serum
15	2 nd immunization		
29*	3 rd immunization	Peripheral blood and serum collection	2 nd post-immune serum
43	4 th immunization		
57*	5 th immunization	Peripheral blood and serum collection	4 th post-immune serum
64*	Completion	Peripheral blood, serum collection	5 th post-immune serum

QC standard: total IgG titer of post-immune serum ≥ 100,000.

Case study-1 sdAb development for soluble protein target (representative data)

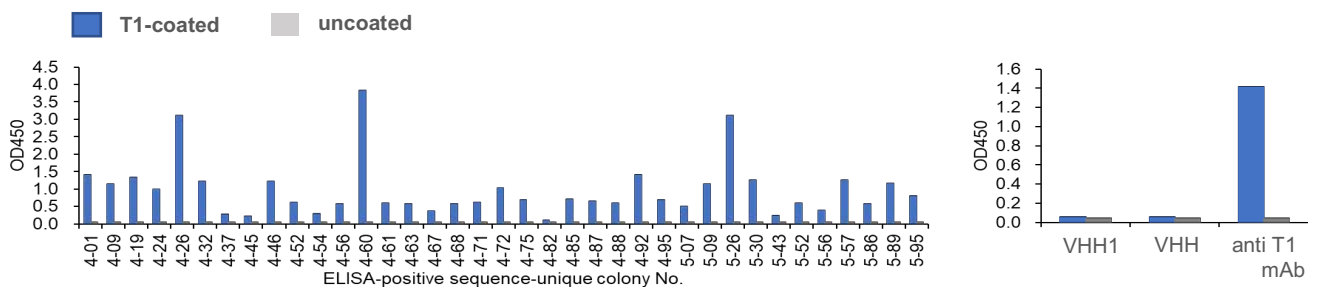
For this case study, one soluble protein target (Target 1 or T1) was provided as an antigen and screening target. Jotbody immunized one alpaca with Target 1 and then develop T1-specific sdAbs.

Titration



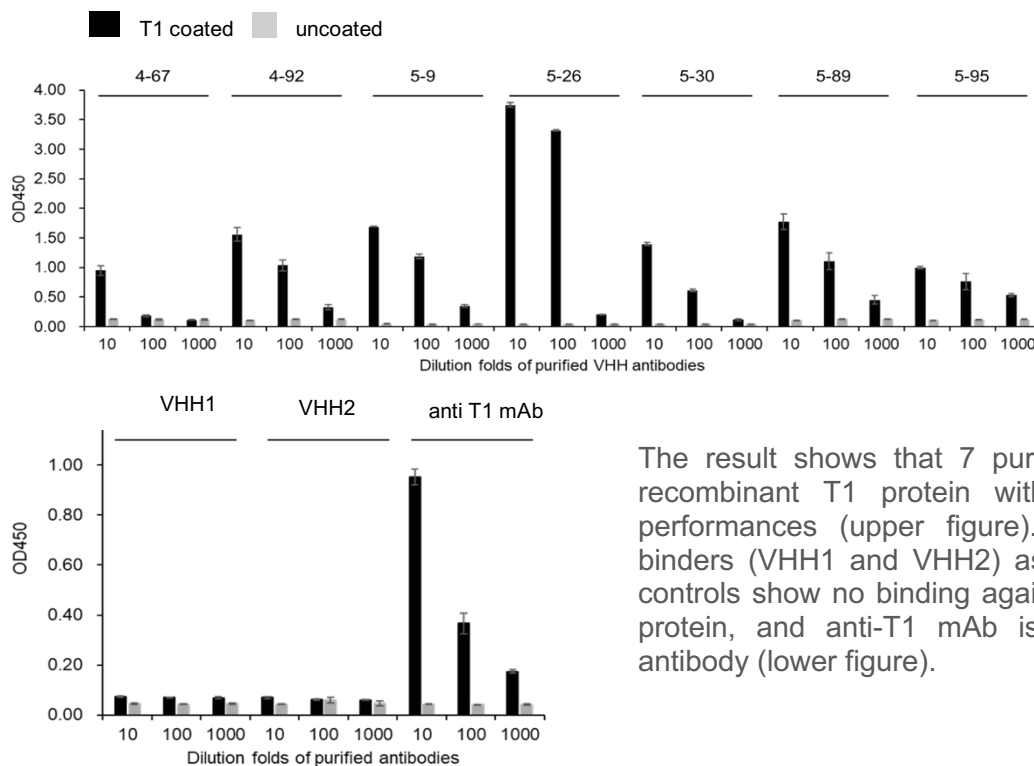
Titration ELISA analysis showing the antigen-specific antibody titer from pre-immune and post-immune alpaca sera collected on the 1st to 5th post-immunization. The 3rd to 5th post-immune sera all reach over 1/100,000.

Periplasmatic ELISA extract against T1



ELISA result from 37 ELISA-positive sequence-unique clones which were identified by sanger sequencing multiple sequence alignment (left figure). ELISA result from two unspecific VHHs non-binders (VHH1 and VHH2) as negative antibody controls and a commercially available anti-T1 mAb as positive antibody control (right figure). The two VHH non-binders shown blank-level background OD450 values similar to uncoated wells, thus suggesting the absence of non-specific binding of the selected VHHs.

Bioactivity validation

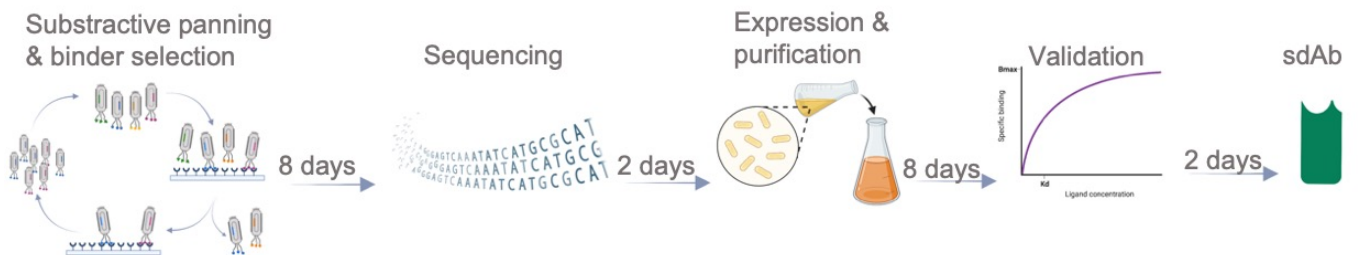


The result shows that 7 purified VHHs bind to recombinant T1 protein with different binding performances (upper figure). Two VHHs non-binders (VHH1 and VHH2) as negative antibody controls show no binding against recombinant T1 protein, and anti-T1 mAb is used as positive antibody (lower figure).

Naïve camelids sdAb phage library

Naïve sdAb Phage Library is a valid alternative to discover sdAb with a short turnaround time and at an affordable price. Naïve libraries are especially recommended for antigens with low immunogenicity, toxic, lethal, transmissible effects, or nonimmunogenic small molecular compounds.

- As fast as 3 weeks to deliver the sdAb, 10 days to deliver the sdAb sequence
- Megadiverse naïve VHH library containing 4.7×10^{11} unique VHH clones as determined by NGS
- Library obtained from 77 healthy, non-immunized camelids (51 Alpacas, 13 Llamas, 13 camels)
- Nearly 100% VHH insert rate



Why are sharks a valid alternative for sdAb discovery?

- vNAR, evolutionarily distant from mammal VH domains, is best for detecting human antigen
- vNAR owns a peculiar paratope structure suitable for enriching the epitope diversity of antigen
- The high concentration of urea in the shark blood forges antibodies that are highly resistant at high pH, temperature and organic solvent

Jotbody's exclusive shark platform

Nurse sharks and Wobbegong sharks are globally used for sdAb discovery and production. Unfortunately, their large body sizes make captivity impossible, and their slow maturity and aggressive temper also make the vNAR's production complicated and expensive. To address these issues, Jotbody developed an innovative shark-derived platform based on ethically farmed home aquarium carpet fish. Jotbody is the only provider of this innovative platform that allows the discovery of high-quality sdAb at an affordable price

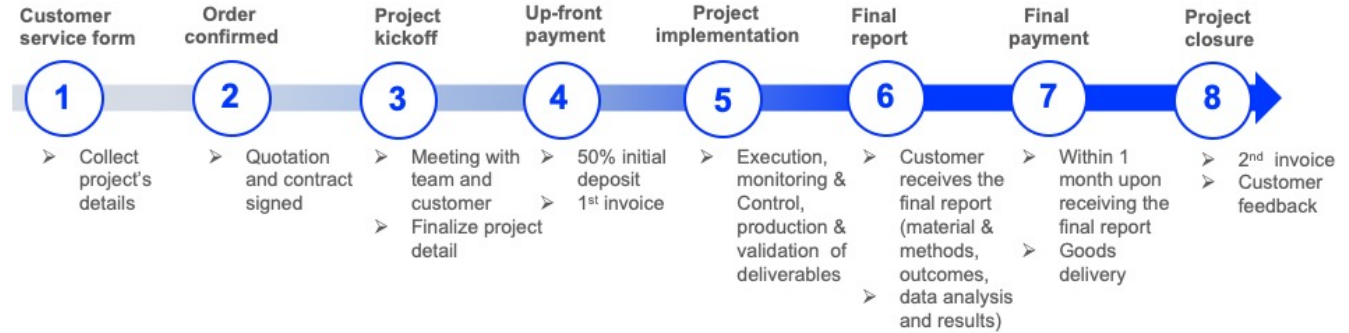
Exclusive know-how



Phase No.	Items	Timeline	Deliverables
I	Antigen evaluation	0~3 weeks	Antigen in proper quantity and quality, QC
II	Animal immunization	<ul style="list-style-type: none"> ~10 weeks for camelids (alpaca, llama, camel) 16~24 weeks for shark 	Sera, PBMC, antibody titer
III	Immune phage display library construction	~3 weeks	Immune library construction, library quality evaluation
IV	Antigen-specific binder screening	~3 weeks	Enriched antigen-specific phages after 3~4 rounds of panning
V	Binder identification and validation	~3 weeks	Sequences of positive clones, purified binders, bioactivity validation
VI	Data analysis and report writing	~1 week	Project final report
total		<ul style="list-style-type: none"> 5~6 months for immune camelids sdAb discovery 6.5~8.5 months for immune sharks sdAb discovery 1.5~2 months for naïve camelids sdAb discovery (no need of Phase II and Phase III) 	

Turnaround time for discovery services

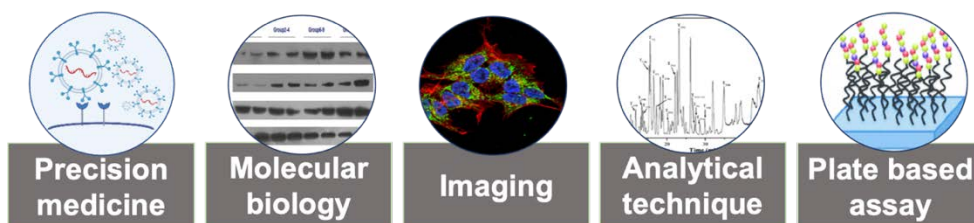
Project implementation & payment plan



SdAb-based research reagents

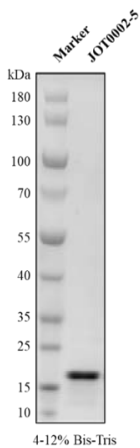
Jotbody offers an extensive range of high-quality sdAb from camelids and sharks. Jotbody develops sdAbs, which are tailored against different antigens and are well validated in a broad range of downstream applications. Every sdAb is thoroughly tested to ensure the highest level of specificity, sensitivity, and reproducibility. To ensure equal, precise quality across batches of the same sdAb, we routinely perform consistency tests to assess batch-to-batch variation.

Given the small size, ~1/10 of conventional antibody, a higher copy number of sdAb interacts with the specific target, with hidden pocket, exhibiting even a better antigen-binding affinity than that of a conventional antibody.



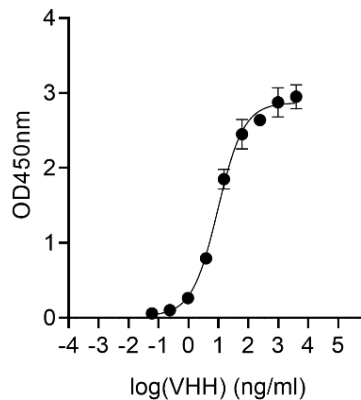
Representative data

SDS-PAGE



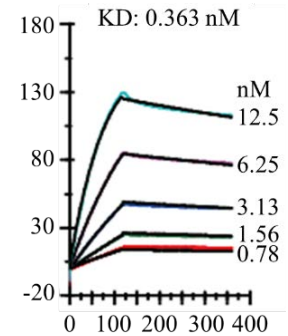
Anti-PD-L1 VHH antibody (JOT0002-5) by 4-12% gradient SDS-PAGE under reducing condition. Protein purity > 95%.

ELISA



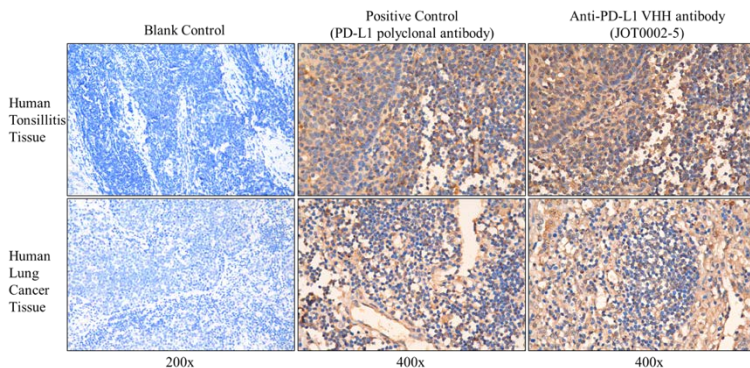
Indirect ELISA shows anti-PD-L1 VHH antibody (JOT0002-5) binding activity against purified PD-L1 protein.

SPR



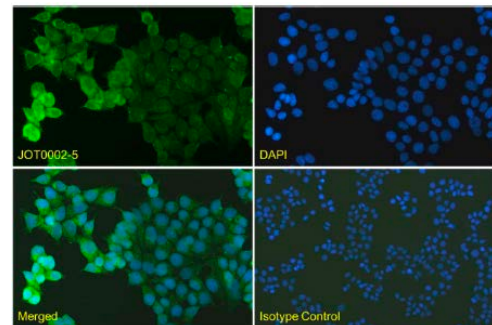
Association/dissociation kinetics between anti-PD-L1 VHH antibody (JOT0002-5) and human PD-L1 captured on CM5 Chip.

Immunohistochemistry (IHC)



IHC analysis of paraffin-embedded human tonsillitis tissue and human lung cancer tissue slides using anti-PD-L1 VHH antibody (JOT0002-5) and positive control (PD-L1 polyclonal antibody (proteintech, # 28076-1-AP)

Immunofluorescence (IF)



Immunofluorescence analysis of paraformaldehyde-fixed MCF-7 cells stained with anti-PD-L1 VHH antibody (JOT0002-5) The isotype control was stained with anti-unknown antibody followed by CoraLite® 488 secondary antibody.

Best Selling

Cat No.	Product Name	Host	Product Type	Application
JOT0001-1	anti-GFP VHH	Alpaca	Primary VHH	ELISA, WB, IP
JOT0001-2	anti-GFP VHH biotinylated	Alpaca	Conjugated VHH	ELISA, IF, WB, IP
JOT0001-3	anti-GFP VHH (Alexa Fluor® 488)	Alpaca	Conjugated VHH	IF
JOT0002-5	anti-PD-L1 VHH antibody	Alpaca	Primary VHH	ELISA, IHC, IF
JOT0005-1	anti-hTNFR1 VHH antibody	Alpaca	Primary VHH	ELISA, WB, IHC, IF
JOT0007-1	anti-SARS-CoV-2 spike RBD VHH	Alpaca	Primary VHH	ELISA
JOT0008-1	anti-Her2 VHH	Alpaca	Primary VHH	IHC, IF
JOT0009-1	anti-EGFR VHH	Alpaca	Primary VHH	IHC, IF
JOT0052-1	anti-GFAP VHH	Alpaca	Primary VHH	WB, IHC
JOT0076-1	anti-mCherry VHH	Alpaca	Primary VHH	ELISA, WB, IF
JOT0121-1	anti-PCSK9 VHH	Alpaca	Primary VHH	ELISA, IHC, IF

FAQs

Does Jotbody provide the antigen?

Yes, we offer protein expression and peptide synthesis services.

How much antigen is needed for sdAb discovery services via immunized animals and naïve sdAb phage library?

We need 2 mg of antigen per camelid immunization and 1 mg per shark immunization. We highly suggest immunizing 1-2 camelids and 6 sharks. This amount of antigen is needed for animal immunization, ELISA and screening. The naïve sdAb discovery service requires 0.5 mg.

What are the antigen requirements?

Purity $\geq 95\%$, Endotoxin Level (EU/ug) ≤ 0.1

Why are immunized libraries better?

Immune libraries develop sdAb of greater affinity and specificity to the antigen due to the natural selection of variant sdAbs during clonal expansion of B-cells in the lymphoid organs of the immunized animals.

When is naïve library screening preferred?

Naïve libraries are recommended for low-immunogenic antigen, toxic, lethal, transmissible effects, or nonimmunogenic small molecular compounds. Moreover, a naïve approach significantly reduces the time and costs required for preparing a new library for any new antigens of interest.

Will there be an absence of immune response after animal immunization?

Once we receive the customer service form, we will perform a detailed investigation to evaluate the feasibility of the project (target, antigen and strategies to improve the immune response are taken into consideration). We will report any potential concerns to our customers before starting the project. So far, we have successfully discovered all antibodies against the target of interest.

How many animals are recommended for immunization per project?

We recommend immunizing 2 camelids or 6 sharks per project.

Does the customer own the right to use and patentability over the discovered sdAb?

Yes, the quotation includes the right to use and patentability over the discovered sdAb.

What are the deliverables?

Our basic package include: pre-immune and post-immune sera, antibody titer assay report, all positive clone sequences, protein validation data and report, 0.1~0.3 mg of purified sdAbs ($>90\%$) (3~5 clones), final project report.

What is considered a new batch for our sdAb Life Science Tool?

For all our nanobody products, a new batch refers to a new lot of the same nanobody by recombinant protein production. Hence, the quality of nanobodies between different batches is consistent. Moreover, we perform tests to confirm the quality of nanobodies for all listed species and applications for subsequent batches.

Any potential biophysical liability e.g aggregation of sdAb made from camel when compared to the one made from mouse or rabbit?

SdAb is resistant to aggregation as nanobody is a monovalent antibody fragment (devoid of light chains) and very stable. SdAb is very small (2~4 nm) and needs fusion format (-Fc fusion) to increase its serum half-life. Humanization is also needed in most cases.

Is the sdAb humanization of nanobody straightforward?

The same strategies for the humanization of a mouse or rabbit-derived mAb can also apply to the humanization of sdAb, such as re-surfacing and CDR grafting.

Key customers



Customer references

"I am writing to express my sincere appreciation for the quality of antibody service you provided. I really appreciate your efficient, gracious service, the level of detail, professionalism, and accountability you have demonstrated on my project, and the way you conduct business as a whole. In short, the service was delivered on time, under budget, and quick resolving any issues that arise.

We look forward to extending our contract with you for years to come and hope you will continue to provide such excellent service to us.

Thank you for the first time in the business with you, and we expect many more. In addition, we will recommend your services to other companies and contacts as well"

Hua Zhang | VP and Chief Scientific Officer, SPH Biotherapeutics

"It is truly a five-star experience regarding order handling, technical support, and product quality. After trying other vendors for nanobody generation, Jotbody is the one I will definitely recommend to my colleagues"

Bill Wong | VP Preclinical Development, InnoRNA

Innovations

- High-affinity anti-EGFP and anti-SARS-CoV-2 vNAR single domain antibodies and use thereof. PCT/CN2021/128297
- Method of Producing Antibody Fragment, CN201811318557.4
- Specific primer pairs designed for single variable new antigen receptor domain derived from IgNAR of Chiloscylidium plagiosum, CN111197074A
- Method of Producing Antibody Fragment, US20190135904

Selected publications

- Bamboo sharks for affordable single domain antibody production. *Front. Bioeng. Biotechnol.* 9:792111.
- Cas9-based local enrichment and genomics sequence revision of megabase-size shark immunoglobulin new antigen receptor loci. *J Immunol.* 2022 Jan 1;208(1):181-189
- The White-Spotted Bamboo Shark Genome Reveals Chromosome Rearrangements and Fast-Evolving Immune Genes of Cartilaginous Fish. *IScience.* 2020; 23(11): 101754
- The novel llama-human chimeric antibody has potent effect in lowering LDL-c levels in hPCSK9 transgenic rats. *Clin Trans Med.* 2020;09, 16

Media coverage research

Pharma Focus Asia <https://www.pharmafocusasia.com/articles/single-domain-antibody>



Jotbody è distribuito in Italia da Prodotti Gianni srl

Via Quintiliano, 30 - Milano - Tel: +39 02 5097 220 - Fax: +39 02 5097 276
Email: ricerca.assistenza@prodottigianni.com - PEC: ricerca@pec.prodottigianni.com

www.ricerca.it