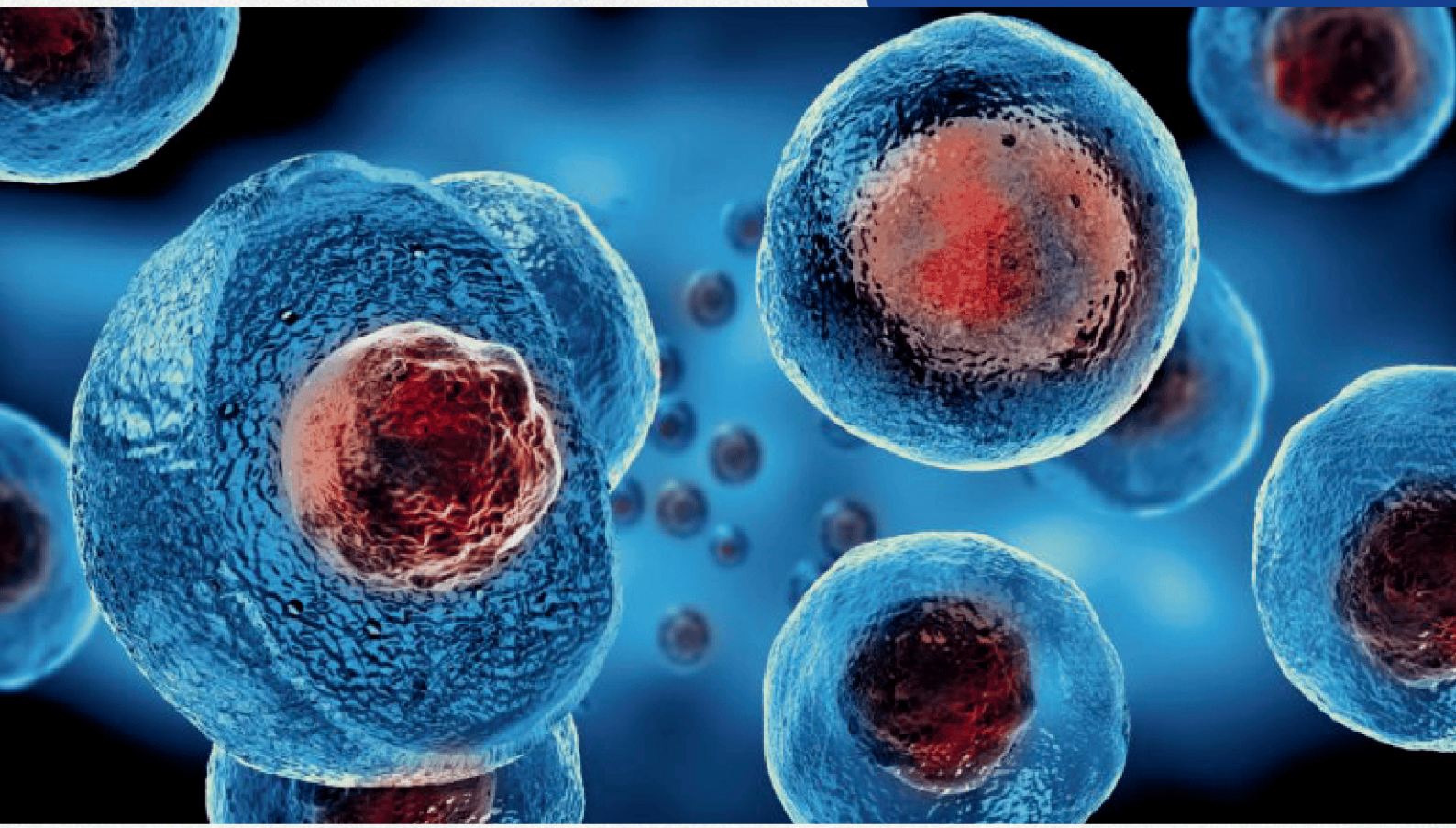
Jetbody

Beyond affinity

First-class nanobody

Promote new progress in life science



Nanobody Service And Product Manuals

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About Jotbody

Jotbody is committed to the development and application transformation of nanobodies, providing high-quality customized development services for nanobodies and high-performance nanobody reagent products for life science workers around the world. Founded in 2020 and incubated by the City University of Hong Kong, the company has an international first-class talent team deeply engaged in the field of biopharmaceutical and life science tools, as well as the world's first patented technology to prepare high-affinity nanobodies using the immune system of small, breeding sharks, solving the problems of complicated production and high development cost of nanobodies.

So far, Jotbody has been funded by Hong Kong Science Park Biomedicine Technology Cultivation Program, Shenzhen Engineering Biotechnology Innovation Center, HK Tech 300 Fund, Hong Kong Innovation and Technology Commission, etc., and has established R&D cooperation with many research institutions at home and abroad.

It serves pharmaceutical companies, biotechnology companies and research institutions from more than 20 countries and regions to jointly promote the development of innovative drugs and scientific tools based on nanobodies.

Jotbody (HK) Limited
(Located in Hong Kong Science Park)

Shenzhen Jotbody Biotechnology Co., LTD
(Located in Shenzhen Engineering Biological Industry Innovation Center)

Nanobody reagent products
(The specific package shall prevail)

Through continuous innovation, Jotbody has developed competitive nanobody reagent products for immunofluorescence, super-resolution imaging, immunoprecipitation , cell sorting, and separation and purification of biological macromolecules in areas with great opportunities and challenges such as neurodegenerative diseases, EVs, and oncology.

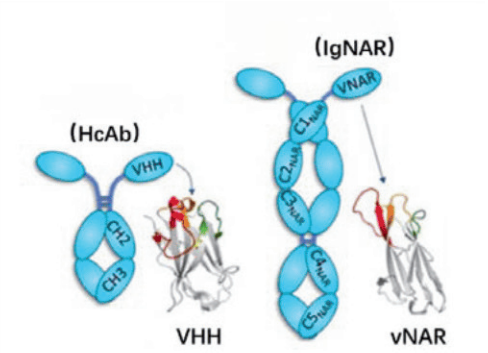
Nanobody related invention patents

The mission of Jotbody is to develop high-performance nanobodies to promote new progress in life science. The vision is to become the most trusted provider of nanobody solutions to promote healthy human life.

About Nanobodies

Nanobody derived from the variable domain of heavy-chain antibody in camels and alpacas (VHH) or the variable domain of new antigen receptor (vNAR) of cartilaginous fish (shark and ray), with a molecular weight of only 12 to 15kDa (2 to 4 nm), is the smallest known antibody fragment.

The unique protein structure of nanobody makes it better than traditional antibody in many properties, and it is more suitable for antibody drug targeted therapy, molecular imaging diagnosis, immunoassay detection and so on.



Nanobodies have advantages over traditional antibodies

- **Small molecular weight, good penetration**

  It can quickly penetrate tissue cells and capture target proteins

- **High specificity and affinity**

  The unique structure can recognize covert epitopes that are not easily recognized by traditional antibodies, and the target binding is better

- **High stability**

  Conformationally stable, high activity can be maintained even at high temperatures and extreme pH

- **Humanization is simple and has low immunogenecity**

  The homology with the human heavy chain is as high as more than 90%, which is suitable for humanization modification

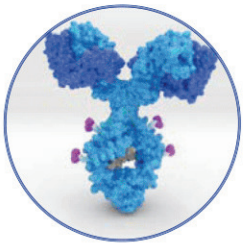
- **Easy for genetic modification and large-scale production**

  The cost of modification and production is low

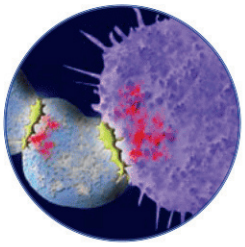
- **High water solubility**

  Improve the utilization rate of drugs

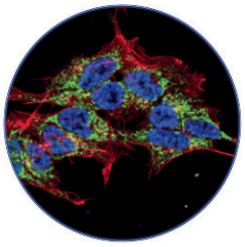
Applications



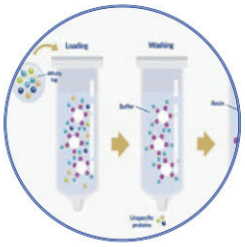
Antibody drug



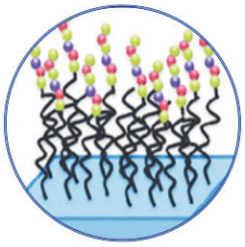
Cell therapy



immunofluorescence



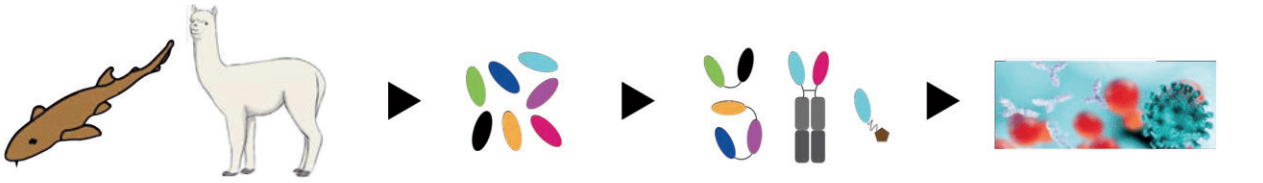
Affinity purification



Analysis and detection

Nanobody service project

- Discovery of nanobodies from camelid immunized libraries
- Discovery of nanobodies from shark immunized libraries
- Discovery of nanobodies from camelid naive libraries
- Discovery of nanobodies from shark naive libraries
- Construction of nanobody libraries
- Humanization of nanobodies based on AI
- Affinity maturation of nanobodies
- Protein expression and purification
- Protein coupling and modification
- Antibody function evaluation
- Design and preparation of multispecific antibodies
- Development of Fc-fused nanobodies



Immunization of camelids or sharks

Screening high-performance nanobodies from high-capacity antibody libraries

Nanobody engineering for different applications.

Production of nanobodies for scientific research, diagnosis and therapy

Service advantage

- **Diverse animal origin**

  Covers camels, alpacas, llamas, sharks

- **Technology platform comprehensive**

  Jotbody has a comprehensive technology platform, including nanobody discovery platform (antigen design and preparation, animal immunity, phage library construction, antibody screening, NGS sequencing, AI-assisted affinity maturation and humanization of nanobody) and protein engineering platform (protein expression and purification, protein modification and modification, protein functional testing).

- **Deliver 20+ sequences**

  Ensure CDR sequence specificity; The delivery can be completed in 3 months at the earliest

- **Independent intellectual property rights**

  The intellectual property rights of the nanobodies generated by the project all belong to the client.

- **Ingenious service**

  Rich project experience, follow up customer needs, solve problems in time , update project progress, and strict QC in every link

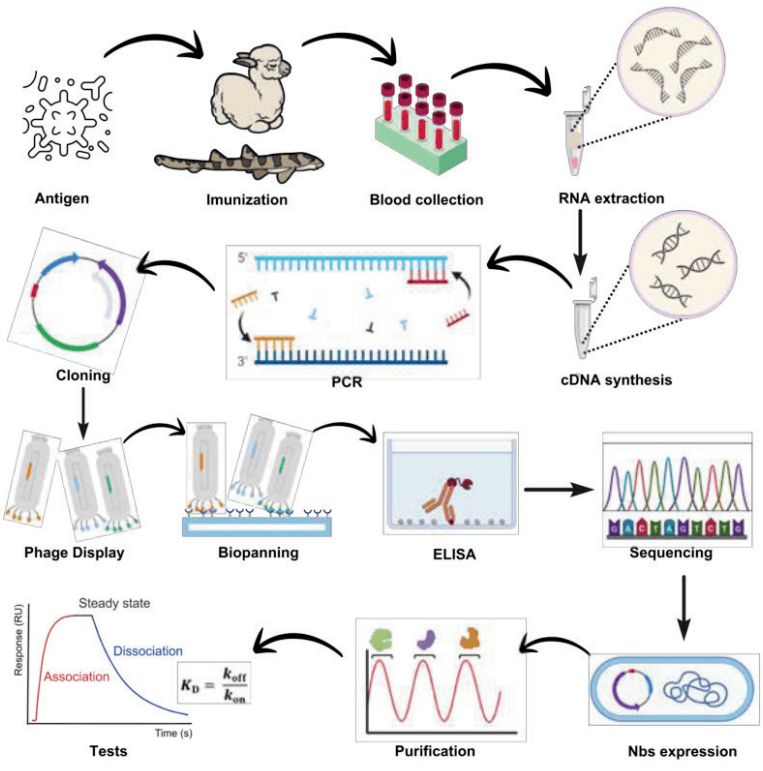
Service contents and cycle of nanobody discovery

Phase	Service content	Service type	Time cycle	Delivery content
I	Antigen preparation	Proteins, peptides, cells, DNA, etc	~3Weeks	Entrust our company to prepare or customer to provide
II	Animal immunization	Alpacas, camels, sharks, etc	~8Weeks	Serum, PBMC, antibody titer report
III	Library construction	Naive library, immune library	~2Weeks	Construction of phage display library and evaluation of library quality
IV	Antibody screening	Solid phase, liquid phase, negative screen, competitive screen, cell screen, etc	~2Weeks	Positive nanobody sequences enriched after panning
V	Antibody evaluation	ELISA/SPR/FC/IF, ect	~3Weeks	Expression and activity validation of nanobody
VI	Report writing	Data analysis of all the above phase	~1Weeks	Final project report
Cycle	Naive library discovery: 1.5~2 months; Immune library discovery: 3~5 months (camel source), 5~7 months (shark source)			

Naive library discovery: from hundreds of healthy non-immune camels (3/5 from alpacas,1/5 from llamas,1/5 from camels), library size 4.7x10^11(as assessed by NGS), VHH insertion rate ~100%, library available for sale.

The advantages of the naive library nanobodies

- The super-large natural library contains 4.7 × 10^11 unique VHH clones (evaluated by NGS), with an approximately 100% VHH insertion rate
- The library is taken from hundreds of healthy, non-immunized camelids (3/5 from alpacas, 1/5 from llamas, and 1/5 from camels)
- Effectively replacing the immunized library, and is particularly suitable for antigens with low immunogenicity, toxicity, or transmissibility.



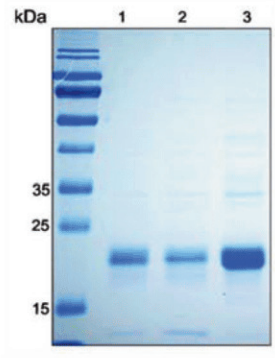
Service contents and cycle of nanobody humanization based on AI

The humanization of nanobodies is crucial for the development of therapeutic nanobody drugs. The sdAb-GPT platform developed by Jotbody provides humanization services of camelid-derived nanobodies based on generative AI, reducing the immunogenicity of non-human antibodies, ensuring that the humanized antibodies have equivalent activity to the original antibodies, and taking into account the improvement of affinity and expression level, as well as the enhancement of stability and solubility.

Phase	Service content	Time cycle	Delivery content
I	Sequence analysis	3 ~ 4 days	Based on GPT generative algorithm, the sequences and structures of the nanobodies were analyzed in depth to identify potential immunogenicity regions
II	Scaffold formation		The human antibody scaffold that best matches the CDR region of the nanobody is generated, which reduces the immunogenicity while retaining the performance of the original antibody as much as possible
III	Structure prediction	1 week	Homology modeling was used to predict the structure and function of humanized antibodies and verify their binding to antigens; Anlaysis of ability and stability; Predict immunogenicity, PTM site, etc
IV	Expression purification	3 ~ 4 days	5~10 humanized antibodies were expressed and purified, and the production performance was evaluated
V	Activity evaluation	1 week	The affinity sequence of antibody molecules ensures that the affinity is not lower than the original at the Biacore detection level 3 times the original antibody
Deliverables: 5~10 humanized nanobody sequences, purified proteins, final project report. Total cycle: 5~6 weeks.			

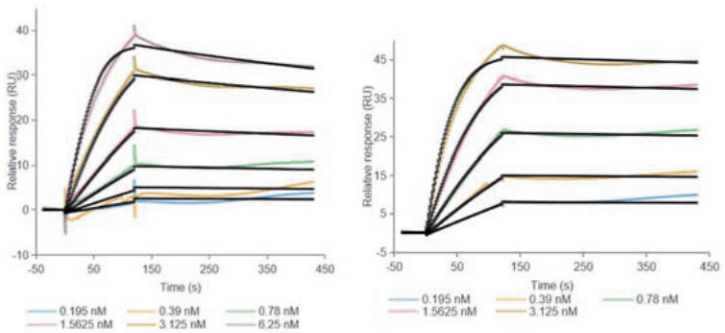
Case study

1. Stability, solubility and yield increasing are considered



Yield
1-2 (PL03): 6.53 mg/L
3 (humanized PL03): 15.39 mg/L

2. The sub-nM affinity of the parental antibody can be increased by nearly 8 times



PL03
ka (1/Ms) = 8.04e+6
kd (1/s) = 5.90e-4
KD (M) = 7.34e-11

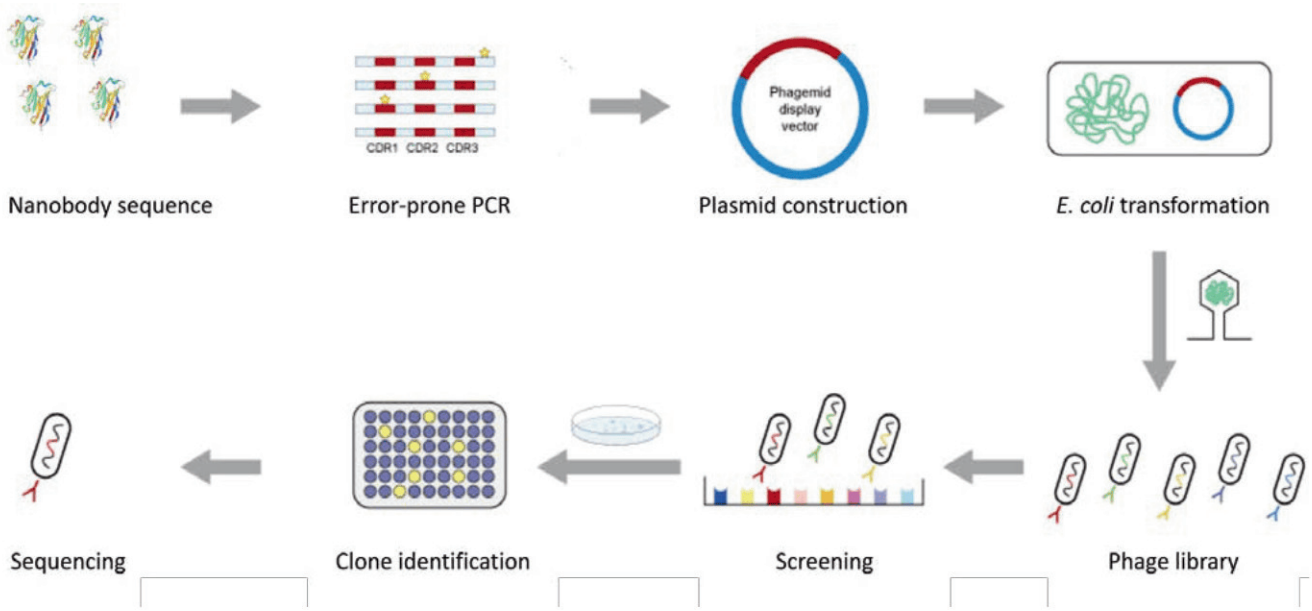
humanized PL03
ka (1/Ms) = 1.15e+7
kd (1/s) = 1.09e-4
KD (M) = 9.48e-12

Service contents and cycle of nanobody affinity maturation

A ffinity maturation refers to the process of optimizing the affinity and specificity of nanobodies through directed evolution in vitro experiments.

Jotbody's nanobody affinity maturation platform uses error-prone PCR to construct a mutant library combined with phage display screening to obtain high-affinity antibody molecules.

Phase	Service content	Time cycle	Delivery content
I	Error-prone PCR	1 week	Design primers based on the original antibody sequence provided by the customer and introduce random mutations through error-prone PCR
II	Construction of phage display library	2 weeks	Construct the phage display library and provide the library quality assessment report
III	Antibody screening	2 weeks	Obtain enriched positive nanobodies after 3 to 4 rounds of panning
IV	Expression and purification	3 weeks	Express and purify 3 to 5 antibodies and evaluate the yield performance
V	Activity verification	3 days	Sort the affinities of antibody molecules. On the Biacore detection level, ensure that the affinities of 2 sequences are more than 5 times higher than that of the original antibody
Deliverables: 3 to 5 optimized antibody sequences, purified proteins, and the final project report (including data analysis of all the above links). Total cycle: ~8 weeks.			



TOP Nanobody Reagents

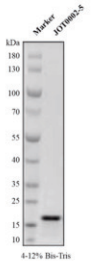
Nanobodies are single-domain antibodies derived from camelids or cartilaginous fish. They are only 15 kDa in size, which is 1/10 of the traditional IgG antibody. They have strong penetrability and high stability, and are especially suitable for immunofluorescence, super-resolution fluorescence imaging, immunoprecipitation, molecular interaction analysis, separation, and purification of biological macromolecules, etc.

Cat No	Product name	Applications
JOT0001-1	anti-GFP VHH antibody	ELISA, WB, IF, IP
JOT0008-1	anti-Her2 VHH antibody	IHC, IF
JOT0009-1	anti-EGFR VHH antibody	IHC, IF
JOT0058-1	anti-Vimentin VHH antibody	ELISA, IHC, IF
JOT0076-1	anti-mCherry VHH antibody	ELISA, WB, IF
JOT0121-1	anti-PCSK9 VHH antibody	ELISA, IHC, IF
JOT0138-1	anti-amyloid-beta 42 VHH antibody	IHC, IF
JOT0002-5-2	anti-PD-L1 VHH antibody (iFluor® 568)	WB, IHC
JOT0052-4	anti-GFAP VHH antibody (Alexa Fluor® 647)	WB, IHC
JOT0142-2	anti-VGLUT VHH antibody (iFluor® 488)	IF
JOT0200 anti-GFP vNAR antibody, clone JP01		WB, IHC

For more details of reagent products, please log in to the official website for inquiry!

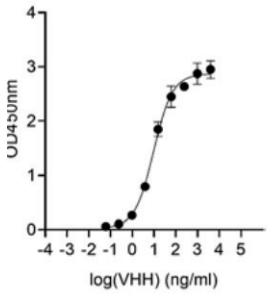
Product data of nanobody research reagents

Purity analysis (SDS-PAGE)



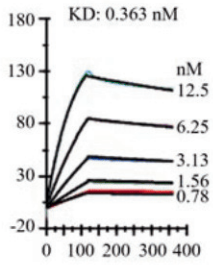
The SDS-PAGE analysis (4-12% gradient gel, reducing conditions) of the PD-L1 VHH antibody (JOT0002-5) shows that the antibody purity is > 95%.

Activity test (ELISA)



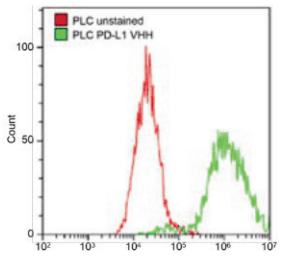
Indirect ELISA shows the binding activity of the PD-L1 VHH antibody (JOT0002-5) to the PD-L1 recombinant protein.

Affinity test (SPR)



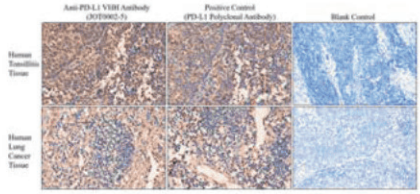
The binding/dissociation kinetics of the recombinant human PD-L1 protein captured by the CM5 chip and the PD-L1 VHH antibody (JOT0002-5).

Flow cytometry analysis (FC)



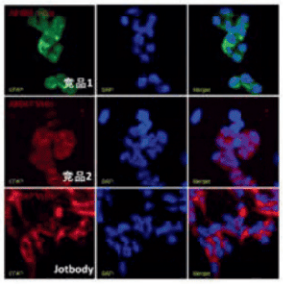
The PLC/PRF/5 cells expressing PD-L1 were stained with the PD-L1 VHH antibody (JOT0002-5) at a 1:50 dilution for flow cytometry analysis.

Immunohistochemistry (IHC)



PD-L1 VHH antibody (JOT0002-5) was used for immunohistochemical analysis of paraffin-embedded human tonsil tissue and human lung cancer tissue sections, and the positive control was PD-L1 polyclonal antibody (a first-line brand).

Immunofluorescence (IF)



GFAP VHH AF647 antibody (JOT0052-4) was used for immunofluorescence analysis of paraformaldehyde fixed U251 cells, and the results showed that the staining effect was better than that of the competitor VHH.

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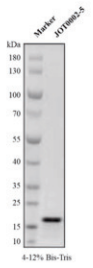

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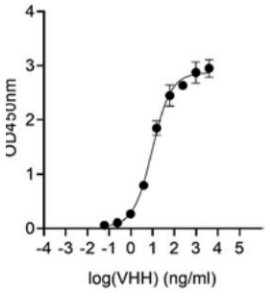

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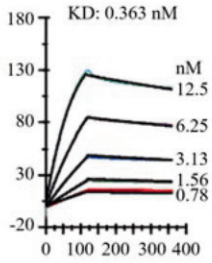

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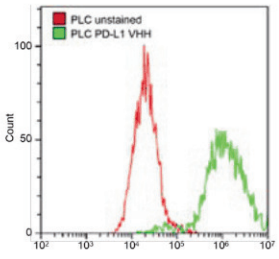

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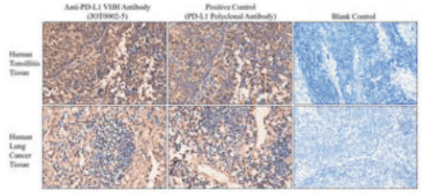

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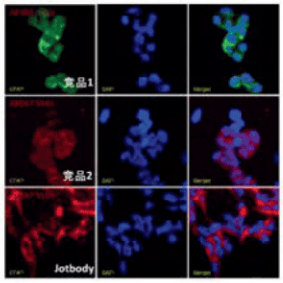

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Common questions

- Under what circumstances is the discovery of nanobodies from the naïve library applicable?

The naïve library is applicable to antigens with low or no immunogenicity, or antigens that are toxic or transmissible. In addition, the naïve library method saves the time and cost required for animal immunization and library construction.

- Why are nanobodies from the immune library better?

After animals are immunized with antigens, B cells in lymphoid organs undergo clonal expansion. At the same time, multiple hypermutations and natural selections are carried out on nanobodies, and the body gradually produces antibodies with stronger affinity and specificity. Therefore, theoretically, nanobodies screened from the immune library are superior in all aspects of performance to those screened from the naïve library.

- Will there be no immune response after animal immunization?

After receiving the customer service form, Jotbody will conduct a detailed investigation to assess the feasibility of the project (considering target characteristics, antigen forms, immunization and antibody screening strategies) to ensure that animals can obtain an effective immune response. Before the project starts, Jotbody will formulate a reasonable project development plan according to customer needs. Up to now, Jotbody has successfully developed antibodies against all the targets of interest to customers.

- How many animals are recommended to be immunized for each project?

For each project, it is recommended to immunize 1-2 llamas or 4-6 sharks.

- Does the customer have the right to use and patent all the discovered nanobodies?

Yes, the quotation includes the customer's right to use and patent the nanobodies discovered in the project development.

- What are the final deliverables?

The final deliverables include: pre-immune and post-immune sera, antibody titer analysis data, library quality assessment data, all positive clone sequences, protein analysis and functional verification data, purified nanobodies, and the final project report.

- Why are Australian alpacas chosen for immunization?

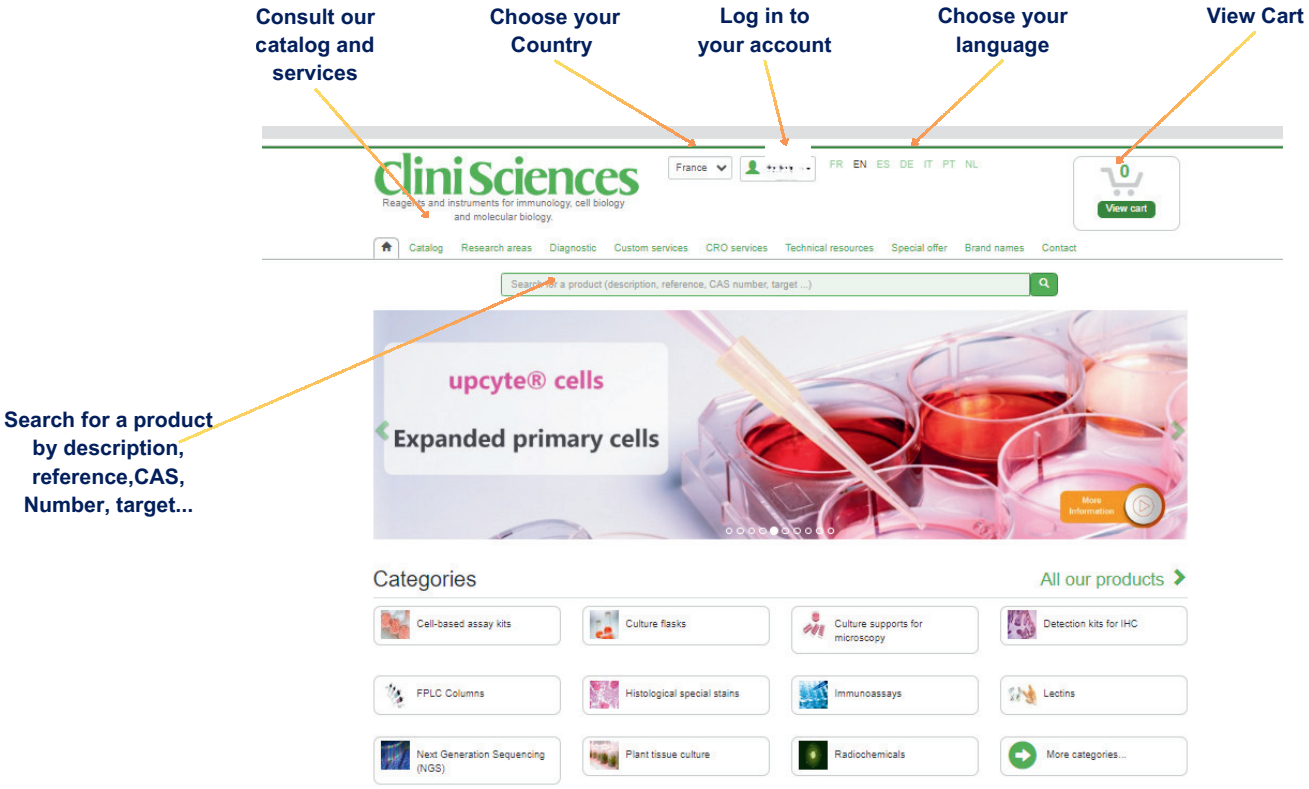
The selection of immunized animals is one of the key factors for the success of immunization. Therefore, healthy and vigorous alpacas with good mental state should be selected for immunization. Australia has globally recognized high standards in animal health and quarantine supervision. Jotbody only selects the best farms in Australia for alpaca immunization, adheres to strict animal breeding standards, ensures that all experimental animals comply with animal welfare ethics, and has a clean immune background.

- Why are sharks an effective alternative animal model for nanobody discovery?

Shark vNAR nanobodies are evolutionarily far from mammalian VH domains and are an excellent choice for detecting human antigens; vNAR has a unique antibody binding site, which can enrich the diversity of antigen epitopes; The high concentration of urea in shark blood contributes to the high stability of vNAR. Jotbody has developed an innovative shark-derived nanobody discovery platform based on a common small shark species that can be aquacultured in an aquarium, which can discover high-quality shark-derived nanobodies at a reasonable cost.

How to navigate through our website?

Homepage: When you first visit our website, you'll land on the homepage. This is where you can find an overview of our services, products, or any important announcements. From here, you can navigate to other sections of the website.



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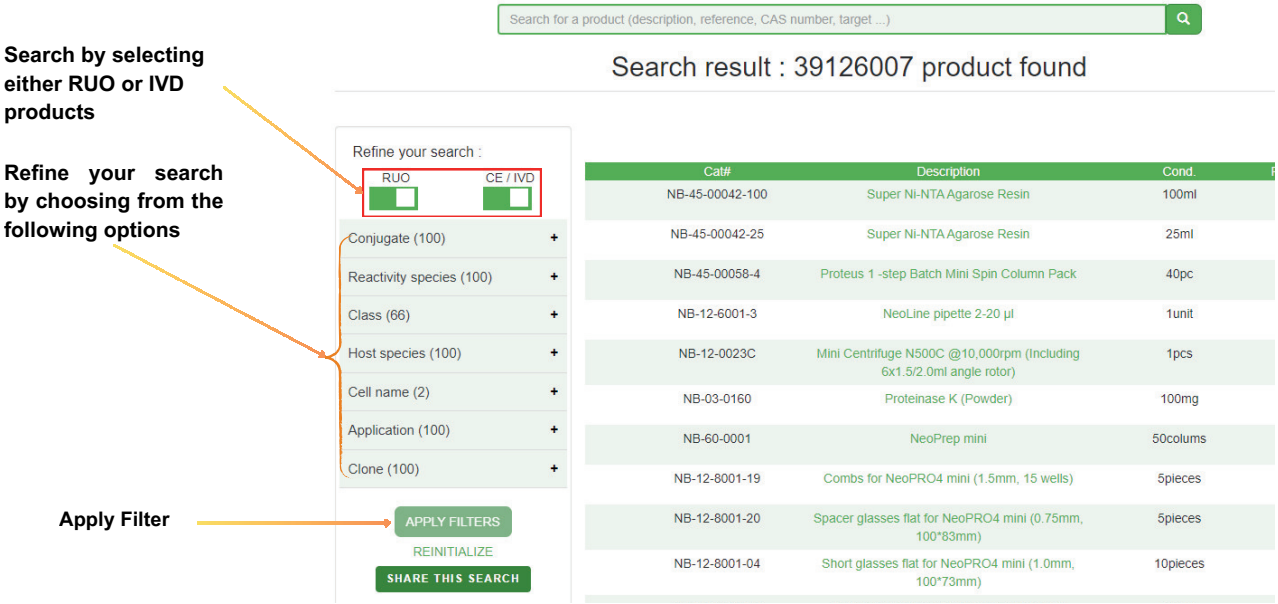

"Remember, if you ever get lost or have questions, don't hesitate to reach out to our customer support team. They're here to help you address any concerns you may have."

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