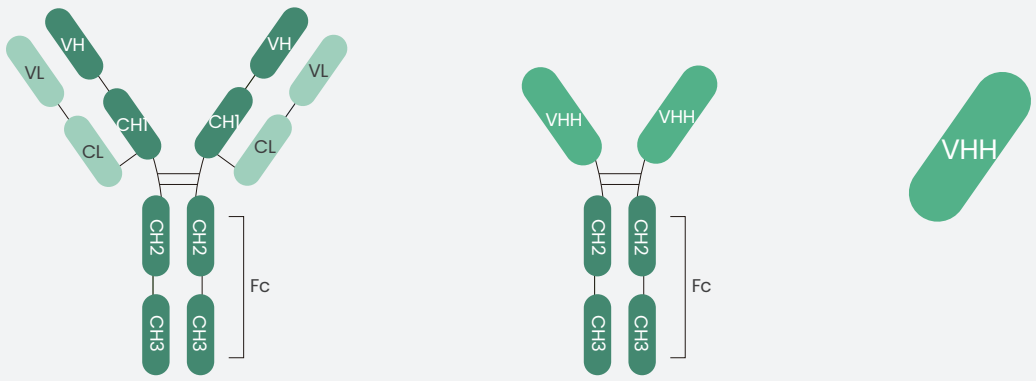
Nanobody Structure, Advantages, Development, and Production

What is a nanobody

Nanobody (Nb) is an artificially designed antibody molecule, also known as a single-domain antibody (sdAb), VHH antibody, or camelid antibody. In 1993, Belgian scientists discovered a heavy-chain antibody (HCAb) in a camel serum sample that was naturally deficient in light chains and had a ~95 kDa molecular weight. The structure includes two constant regions (CH2 and CH3), a hinge region, and a variable heavy-chain domain (VHH). From this, a single-domain antibody containing only a VHH domain was obtained. The crystal structure of VHH antibody is an oval shape of 4 nm×2.5 nm×3 nm in size, and its molecular weight is only 1/10 of conventional antibodies, which is 12–14 kDa, making the former the smallest intact antigen-binding fragment. Hence, the term "nanobody" was derived (Fig. 1).



a. Conventional antibodies b. Heavy-chain antibodies c. VHH

Fig. 1 Schematic illustration of the conventional antibodies, heavy-chain antibodies, and nanobodies

Nanobody Structure

Nanobodies (VHH) possess the same domains as traditional antibody VH, which includes four conserved framework regions (FR1/2/3/4) and three complementarity-determining regions (CDR1/2/3) (Fig. 2). There are four highly conserved hydrophobic amino acid residues (V42, G49, L50, and W52) of FR2 in VH of the conventional antibodies. However, in VHH antibodies, these residues are replaced with hydrophilic amino acid residues (F42 or Y42, E49, R50, and G52), which increases the solubility of nanobodies. In addition, CDR3 of VHH is a bit longer than VH and can form a convex structure, enhancing the ability to recognize hidden tumor antigenic epitopes.

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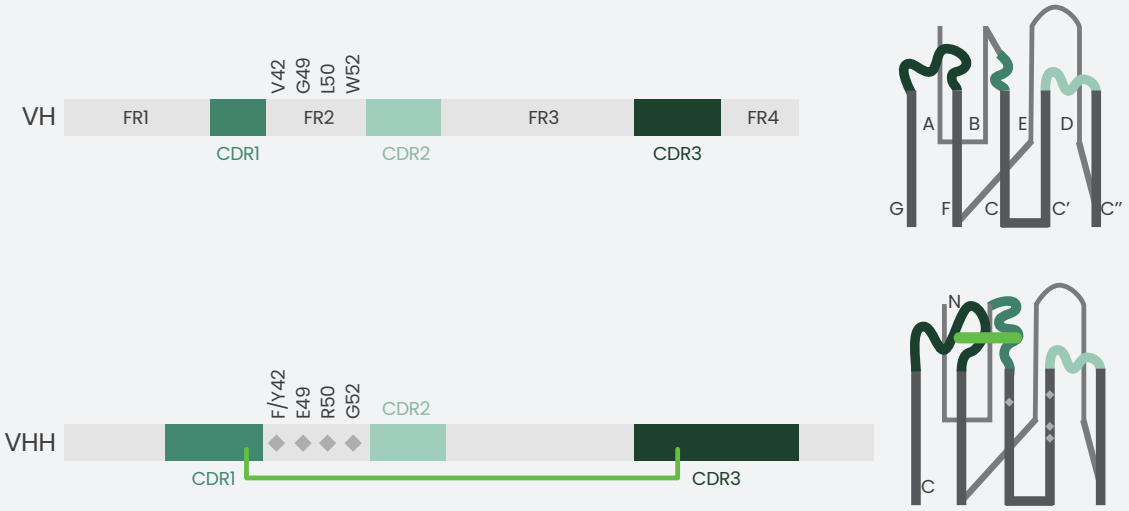

Fig. 2 Schematic illustration comparing VH with VHH.

Table 1. Antibody vs Nanobody

Feature	Antibody	Nanobody
Immunogenicity	High	Low
Molecular weight	150 kDa	12–14 kDa
Half-life	Long	Short
Tissue penetration	Low	Strong, it can cross the blood–brain barrier
CDR3 length	10 amino acid residues on average	16–24 amino acid residues
Recognition site	Harder to identify hidden sites	Easier to identify hidden sites
Stability	Prone to degradation and changes in temperature and pH	High stability. Tolerant to temperature, pressure, and pH changes
Antibody expression	Mammalian expression	Mammalian or microbial expression
Production cost	Mammalian or microbial expression	Low
Engineering modification	"Y"-shaped structure, not easy to modify	Simple structure that can be easily modified

Nanobody Types

Nanobodies can be classified into several different types, such as monovalent nanobodies, bivalent nanobodies, bispecific nanobodies, multivalent nanobodies, and nanobodies fused with other fragments (Fig. 3).

Bivalent or multivalent nanobodies possess two or more VHH structures that recognize the same epitope of the antigen and have higher affinity than monovalent nanobodies with no impact on their pharmacokinetics and targeting ability. Bispecific nanobodies possess two different VHHs that bind two different antigens, or different epitopes on the same antigen, thereby offering stronger antigen recognition than monovalent nanobodies.

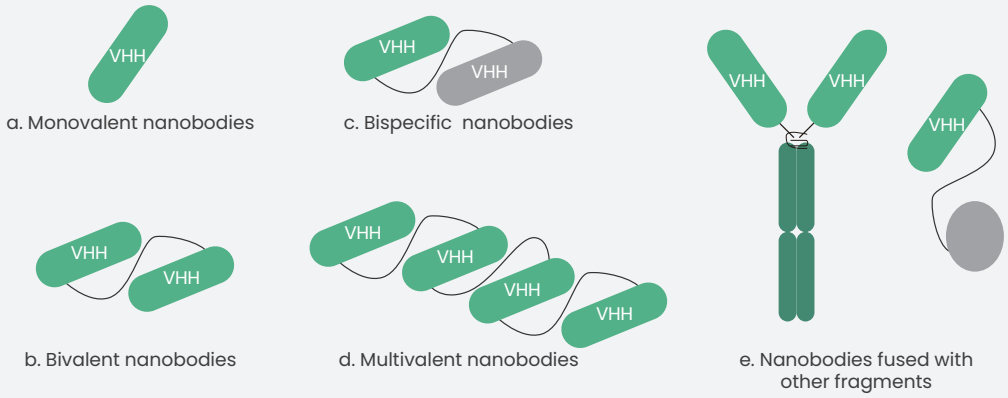

Fig. 3 Types of nanobodies

Unlike conventional antibodies, nanobodies do not have the Fc segment and cannot produce cytotoxic effects such as ADCC/CDC. Therefore, the VHH antibody can be expressed in fusion with the Fc segment to construct Fc-VHH fusion proteins for increasing ADCC and CDC activities. Compared with conventional antibodies, these formats of nanobodies can be widely applied to the treatment of various diseases.

Nanobody Advantages and Limitations

The molecular weight of is only 10% of the conventional antibodies and they retain the complete antigen-binding ability of HCAbs. Owing to their high specificity, good affinity, and stability, nanobodies are widely applied in biochemical mechanisms, structural biology, diagnosis, and tumor treatment.

• Advantages of Nanobodies

Simple structure, easy to develop and express

Owing to the possession of only a variable heavy-chain domain, a simple structure, and no post-translational modifications, VHH antibodies can be genetically engineered into different formats. They can also be expressed and produced in prokaryotic and eukaryotic cells on a large scale with low costs.

High stability and strong affinity

Nanobodies are extremely stable and resistant to protease degradation. They can be stored for long periods at >90°C and can be effectively renatured after thermal denaturation. Nanobodies can maintain their biological activity under high pressure, strong acid, and strong base conditions. Although a VHH antibody lacks a light chain, it has a longer CDR3 and can bind tightly to antigens; therefore, the affinity of nanobodies is comparable to that of conventional antibodies.

High targeting ability and tissue penetration

The long CDR3 of nanobodies can form a convex structure that can penetrate deep into the antigen and recognize hidden epitopes. In addition, the hydrophobic residues in the FR2 region of nanobodies are replaced with hydrophilic residues, which enhance solubility and tissue penetration, and can effectively penetrate tumor tissues and the blood–brain barrier.

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Low immunogenicity and ease of humanization

Nanobodies have low immunogenicity as they have a small molecular weight and fewer epitopes available to bind to antigens. With a high sequence homology of >80% with human heavy-chain antibodies, nanobodies are suitable for engineering into humanized antibodies, which reduces the immunogenicity of VHH antibodies as therapeutic drugs. As a provider of antibody humanization service, Sino Biological can complete the humanization of nanoantibodies in as fast as 3-4 weeks through complementarity-determining region (CDR) grafting technology and computer-aided molecular modeling, with a 100% success rate.

• Limitations of Nanobodies

The half-life of nanobodies is short, which limits their clinical applications. However, by fusion with antiserum albumin or Fc segment, the half-life of nanobodies in the blood can be extended.

Clinical Applications of Nanobodies

Considering their excellent performance, nanobodies have been widely applied in molecular imaging and diagnostics, drug delivery, tumor immunotherapy, cell therapy, and other research fields. Notably, nanobodies have demonstrated excellent value and prospects for application in the diseases of the central nervous system, circulatory system, tumors, and infectious and inflammatory diseases. Especially in the field of tumor therapy, a variety of therapeutic agents such as bispecific nanobodies, nanobody-drug-conjugate (NDC), CAR-T, CAR-NK, and targeted radionuclides have been developed. The continuous research and development of these therapeutic approaches have brought new hope to patients.

As of July 2023, at least four nanobody drugs have been approved for marketing worldwide. Caplacizumab (trade name Cablivi®), developed by Ablynx, is the world's first nanobody drug applicable for the treatment of adult patients with acquired thrombotic thrombocytopenic purpura (aTTP); this drug was approved for marketing on August 31, 2018, by the European Medicines Agency. The CAR-T cell product developed by Legend Biotech, CARVYKTI (generic name Ciltacabtagene Autoleucel) is a unique bivalent nanobody design; it is also the first FDA-approved VHH-based CAR-T product application for relapsed or refractory multiple myeloma treatment. Ozoralizumab, another nanobody drug developed by Ablynx, is a humanized, trivalent, bispecific nanobody consisting of two anti-human TNFα nanobodies and one anti-human serum albumin nanobody, which was approved for marketing on September 26, 2022, in Japan. Envafolimab developed by Alphamab Oncology, belonging to the PD-L1 single-domain antibody Fc fusion protein, was approved for marketing in November 2021 in China. In addition, presently, more than 20 nanobody-related drugs have entered the clinical stage.

Clinical research progress of nanobody drugs

Drug name	Type	Target	Disease	Phase
Caplacizumab	VHH	VWF	• Thrombotic thrombocytopenic purpura (aTTP)	Approved for marketing
Ozoralizumab	Trivalent bispecific VHH	TNF, TNF-α	• Autoinflammatory diseases • Rheumatoid arthritis	Approved for marketing
ciltacabtagene autoleucel	Bispecific VHH	APRIL	• Multiple myeloma	Approved for marketing
Sonelokimab	VHH	IL-17	• Purulent sweat disorder • Autoinflammatory diseases • Psoriasis • Psoriatic arthritis	Phase 2
SAR-442970	VHH	OX40L, TNF, TNF-α	• Purulent sweat disorder • Immune dysregulation diseases • Autoinflammatory diseases	Phase 1

Drug name	Type	Target	Disease	Phase
ALX-0141	Bispecific VHH	ODFR, RANKL	• Bone diseases • Metastatic malignant tumors of bone or bone marrow • Postmenopausal osteoporosis	Phase 1
V-565	VHH	TNF-α	• Crohn's disease • Ulcerative colitis	Phase 2
ZL-1102	VHH	IL-17	• Autoinflammatory diseases • Psoriasis	Phase 1
SAR-443765	VHH	IL-13, IL-13R, TSLP	• Asthma • Immune dysregulation diseases • Autoinflammatory diseases	Phase 1
LQ-036	VHH	IL-13R, IL-4R	• Asthma	Phase 1
Envafolimab	VHH	PD-L1	• Solid tumors • Malignant tumors • Endometrial cancer • Human immunodeficiency virus disease • HIV-1 infection • Hepatitis B virus • Hepatocellular carcinoma • Metastatic colorectal cancer • Metastatic non-small cell lung cancer • Metastatic renal cell carcinoma • Metastatic malignant tumor of the stomach • Non-small cell lung cancer • Sepsis • Sepsis with septic shock • Soft tissue sarcoma	Approved for marketing
SAR-443726	VHH	IL-13, IL-13R, OX40L	• Atopic dermatitis	Phase 1
NM-01	VHH	PD-L1	• Malignant tumor • Metastatic non-small cell lung cancer • Non-small cell lung cancer	Phase 2
BI-836880	Bispecific VHH	ANGPT2, ANGPTL2, VEGF, VEGFA, VEGFR	• Solid tumors • Malignant tumors of the anus or anal canal • Malignant tumor of the gastrointestinal tract • Metastatic non-small cell lung cancer • Wet age-related macular degeneration	Phase 2
KN-044	VHH	CTLA4	• Solid tumors • Malignant tumor	Phase 1
BI-655088	VHH	CX3CR1	• End-Stage Renal Disease (ESRD)	Phase 1
HLX-53	VHH	TIGIT	• Solid yumors • Malignant lymphoma	Phase 1
LCAR-AIO	CAR-T	CD19, CD20, CD22	• Acute lymphocytic leukemia • B-cell lymphoma	Phase 1
SC-DARIC33	CAR-T	CD33	• Acute myelogenous leukemia	Phase 1
LAVA-1207	Bispecific T cell engager antibody	GCP2, PSMA, TCR	• Hormone-resistant prostate cancer • Solid tumor	Phase 2

Recommended Services

Anti-idiotype Antibody Production Services

Nanobody Development and Production Platform

Nanobodies are very promising next-generation therapeutic antibodies that have received increasing attention from research organizations and pharmaceutical companies. To support the early discovery of nanobody drugs, Sino Biological independently developed a nanobody development platform, that utilizes the phage antibody library technique, and has successfully developed several nanobody candidate molecules. Additionally, a variety of nanobody formats have been developed and produced using our high-throughput nanobody production platform, including monovalent, multivalent, or multi-specific VHH, which can meet a variety of customized needs of our customers.

Nanobody Development Services

Unlike the classical hybridoma technique applied to the development of monoclonal antibodies, the entire nanobody development process includes alpaca immunization, phage library construction, nanobody screening, expression, purification, and validation stages. After alpaca immunization, B lymphocytes are isolated from the peripheral blood, after which total RNA is extracted and reverse-transcribed to cDNA; this cDNA is used as a template for PCR amplification to obtain diversified nanobody gene fragments, which are then ligated into vectors to construct nanobody phage libraries. Subsequently, multiple rounds of panning steps are performed to obtain antigen-specific nanobodies, followed by sequencing, expression, and validation (Fig. 4).

Sino Biological has established a nanobody development platform to provide a one-stop custom nanobody service. It mainly includes antigen design and preparation, alpaca immunization, library construction, panning, monoclonal identification, sequencing, and activity analysis. The obtained nanobodies need to be further humanized to reduce their immunogenicity and achieve the best therapeutic effect. The nanobody humanization service provided by Sino Biological, can humanize alpaca nanobodies by using complementarity-determining region (CDR) grafting technology and computer-aided molecular modeling to ensure a high degree of successful humanization (>95%) and 100% success rate. We have successfully delivered many nanobody development projects and provided *in vitro* efficacy evaluation services to meet application scenarios such as nanobody druggability assessment and biological activity determination.

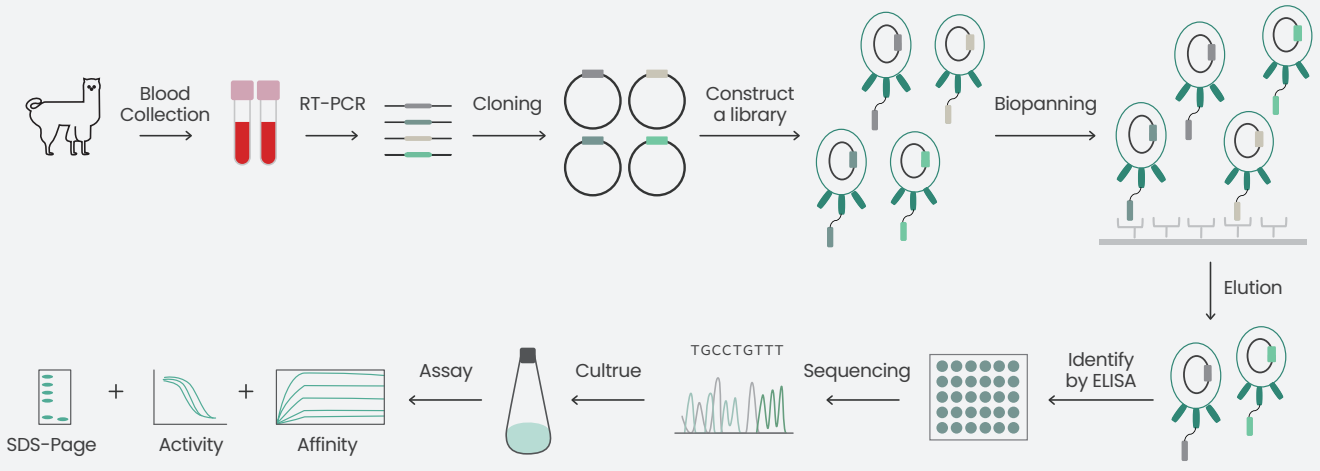

Fig. 4 Nanobody development by phage display technique

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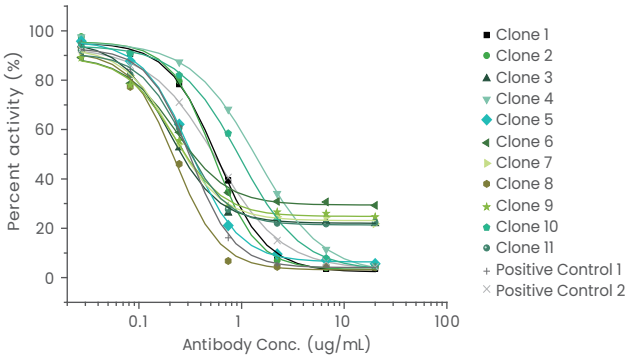
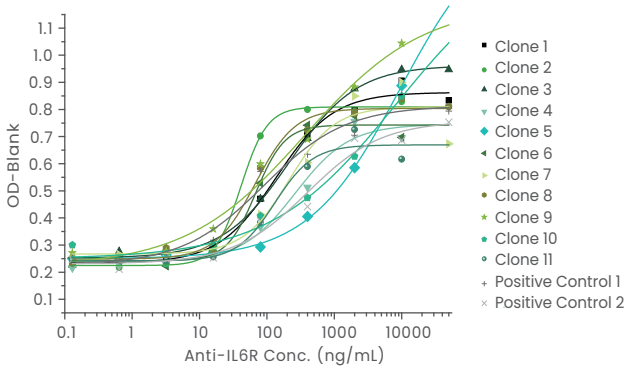
Case Study of IL-6R Nanobody Development

Through our nanobody platform, Sino Biological has obtained 11 anti-IL6R nanobodies by competitive screening and verifying their blocking activity and cell-binding activity. Finally, 2 clones have been screened as candidate molecules to use in the next step of development. Specific data are shown below.

Blocking Acitivity



Cell-binding Activity

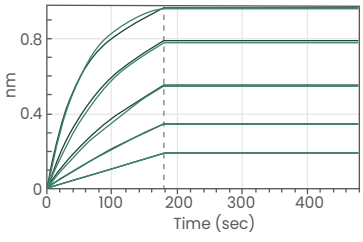


IC_{50} and EC_{50} Data

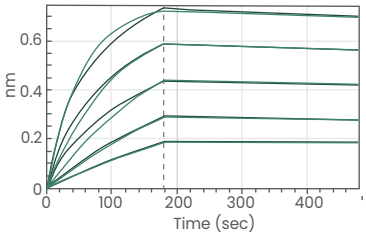
Sample ID	IC_{50} (μg/mL)	EC_{50} (μg/mL)
Clone 1	0.5758	0.136
Clone 2	0.5306	0.041
Clone 3	0.1967	0.217
Clone 4	1.4049	0.266
Clone 5	0.3182	/
Clone 6	0.2268	0.068
Clone 7	0.2134	0.212
Clone 8	0.2258	0.067
Clone 9	0.2201	0.369
Clone 10	0.9702	/
Clone 11	0.2701	0.131
Control 1	0.319	0.09
Control 2	0.5663	0.401

Affinity Determination

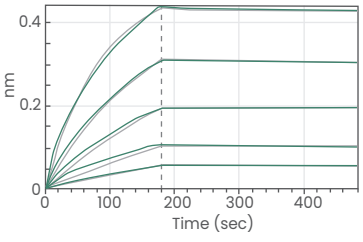
Clone 2: K_D=9.37E-11M



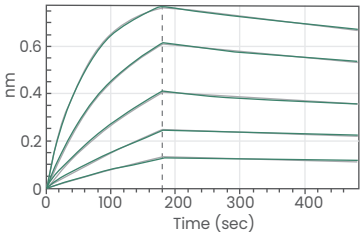
Clone 8: K_D=4.63E-10M



Control 1: K_D=2.18E-10M



Control 2: K_D=1.61E-09M



• High-throughput Nanobody Production Services

Powered by professional high-throughput primer synthesis and vector construction, our high-throughput recombinant antibody production platform can perform high-throughput antibody expression. In addition to full-length IgG antibodies, Sino Biological can produce various formats of antibodies such as multivalent VHHs and VHH-Fc fusion-type nanobodies with high success rates to meet the different individual needs of customers.

The specific process is as follows: After obtaining the nanobody sequence library, the nanobody expression library is established by high-throughput primer synthesis and vector construction. Then, the plasmids are transfected into HEK293 cells in shake flasks. Nanobodies are then purified by one-step Protein A or Ni affinity chromatography with >90% purity. The purified nanobodies are functionally validated before scaling up the production (Fig. 5).

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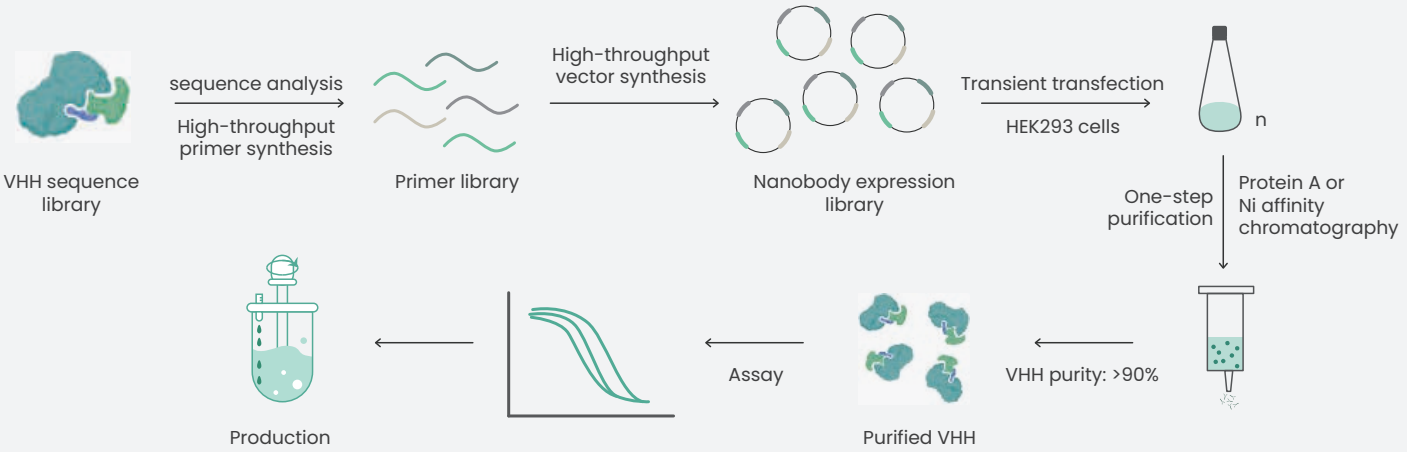

Fig. 5 High-throughput nanobody expression platform

References

1. Jovčevska I, Muyldermans S. The Therapeutic Potential of Nanobodies. BioDrugs. 2020 Feb;34(1):11-26.

2. Naidoo DB, Chuturgoon AA. Nanobodies Enhancing Cancer Visualization. Diagnosis and Therapeutics. Int J Mol Sci. 2021 Sep 10;22(18):9778.

3. Van Audenhove I, Gettemans J. Nanobodies as Versatile Tools to Understand, Diagnose, Visualize and Treat Cancer. EBioMedicine. 2016 Jun; 8:40-48.

4. Panikar SS, Banu N, Haramati J, Del Toro-Arreola S, Riera Leal A, Salas P. Nanobodies as efficient drug-carriers: Progress and trends in chemotherapy. J Control Release. 2021 Jun 10; 334:389-412.

5. Liu X, Balligand T, Carpenet C, Ploegh HL. An armed anti-immunoglobulin light chain nanobody protects mice against influenza A and B infections. Sci Immunol. 2023 Jun 23;8(84):eadg9459.

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