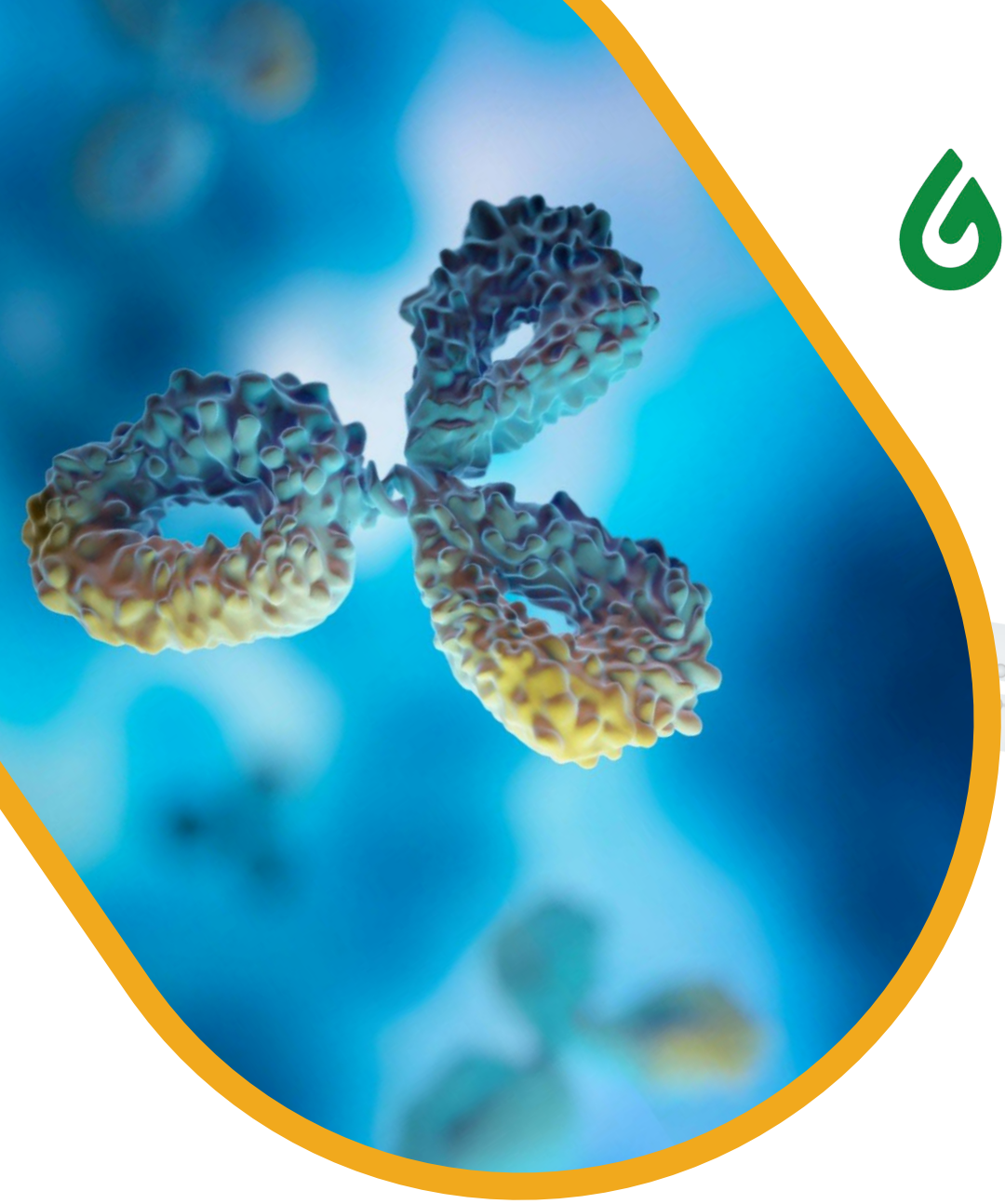


GenScript
Make Research Easy

Applications of Single-chain Antibody and Nanobody in Antibody Drug Development and CAR-T Therapy

- Antibody Discovery
- Antibody Purification
- CAR-T Cell Characterization and Isolation

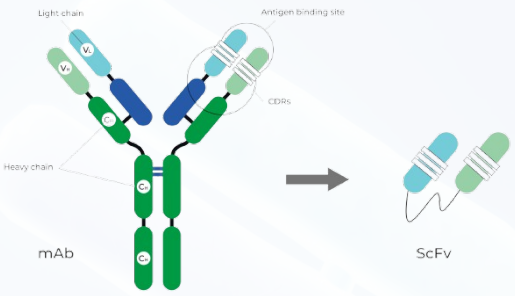
CONTENTS

INTRODUCTION

Single-chain fragment variable (scFv) and nanobody (also known as VHH) are both genetically engineered antibodies. Due to their small molecular weight, strong penetration, short half-life and low immunogenicity, these antibodies play an important role and have broad application prospects in disease prevention, diagnosis and treatment.

What is scFv?

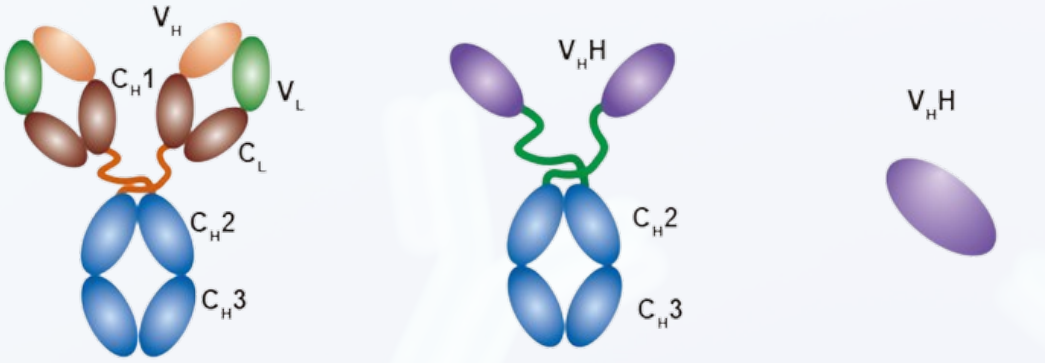
A single chain fragment variable (scFv) is a type of recombinant antibody. It is approximately 25 kDa and consists of the variable regions of the heavy (VH) and light (VL) chains of an antibody, which are connected by a flexible peptide linker (such as G4S linker or whitlow/218 linker)[1]. scFvs have several advantages, including their small molecular weight, strong penetration, and high specificity. They play crucial roles in targeted therapy, imaging diagnosis, and biological detection. Importantly, in the field of CAR-T, scFvs can act as the antigen recognition domain of CAR-T cells and determine the targeting ability of these cells.



GenScript has developed a series of high-quality anti-scFv antibodies to support your research in the field of scFv-based antibody drugs, bispecific antibodies, and CAR-T.

What is VHH?

Members of the Camelidae family, including llamas, alpacas, and camels, produce two types of IgG antibodies. One type is the conventional IgG1 (MW150 kDa) with two heavy chains and two light chains. The other type, IgG2 and IgG3 (MW90 kDa), lacks light chains and one constant domain of the heavy chain (CH1 domain). These are called heavy chain antibodies (HCAbs). The variable domain of HCAbs is known as a single domain antibody, also called sdAb, nanobody, or VHH, and is the smallest antigen-binding entity[2].



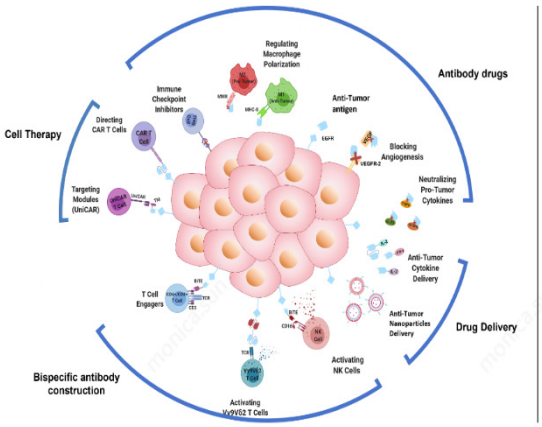
Conventional IgG (Camelid Ig G1)

Camelid Ig G2 or Ig G3

Single domain antibody (sdAb)

Advantages of scFv and VHH Applications

VHH nanobodies and single-chain fragment variables (scFv) are genetically engineered antibodies with advantages such as small size, strong penetration, short half-life, low immunogenicity, and easy engineering. They excel over full-length antibodies in cell therapy, bispecific antibody construction, solid tumor treatment, and drug delivery. Additionally, they are widely used in viral infection, biological monitoring, biosensors, and basic research. As research progresses, scFv and VHH are expected to play an increasingly significant role in medicine, agriculture, and industry.



Comparison of scFv and VHH Structures and Physicochemical Properties[3]

Nature	scFv	VHH
Size	28-35 KDa	12-15 KDa
Blood half-life	1h	1h
Source	Mice, rats, etc.	Camelidae
Development time	Shorter	Longer
Water Solubility	Good water solubility (+)	Good water solubility (+++)
Gather	Easy to aggregate	Almost no gathering
Stability	Moderate stability (+)	Excellent stability (++)
Resistant to extreme environments	Not resistant to high temps. & extreme pH	Stable at high temps. & extreme pH
Tissue penetration	Strong tissue penetration (++)	Better tissue penetration (+++)
Affinity	High affinity (+)	Higher affinity (++)
Cost of production	Low	Lower
Engineering Transformation	Easier to modify (+)	Very easy to modify (+++)
Epitope diversity	Consistent with traditional antibodies	New epitopes available
Hidden epitope recognition	Difficult to identify hidden epitopes	Can identify hidden epitopes
Expression system	Prokaryotes, yeast, mammals	Prokaryotes, yeast, mammals

Note: "+" represents the degree of the descriptive item. The more "+" signs present, the higher the degree.

Overview of scFv and VHH Applications

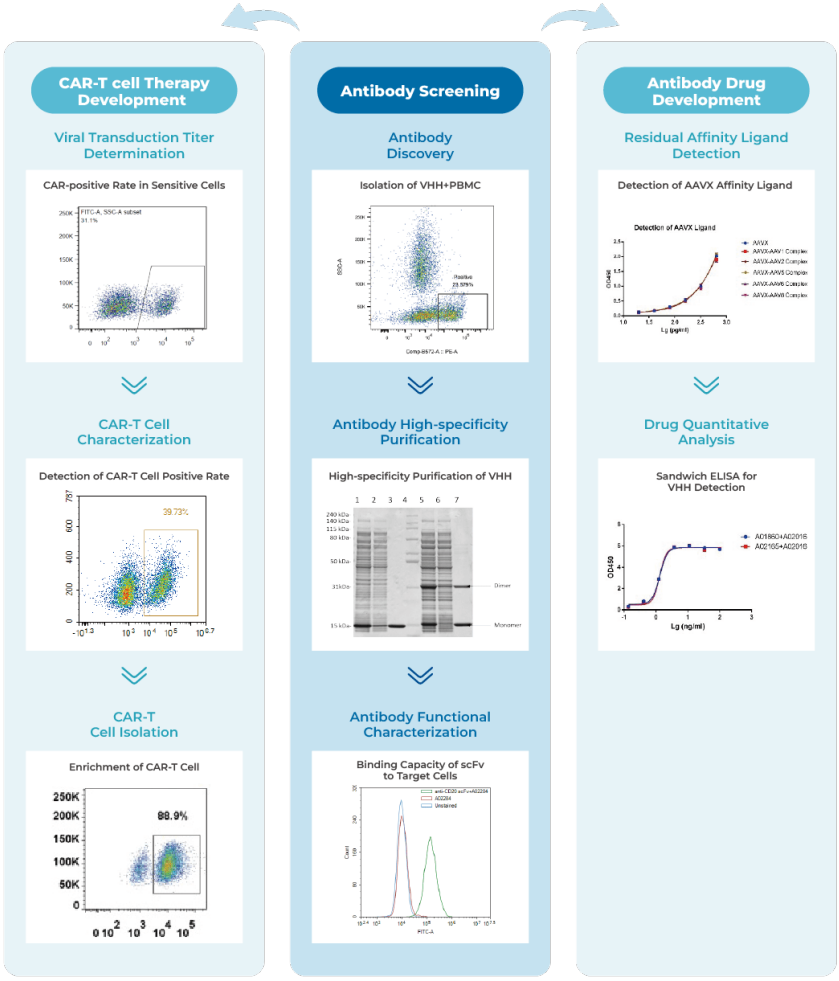
Bottlenecks of existing scFv and VHH research tools and reagents

scFv and VHH have broad applications and are attracting increasing interest from research institutions and pharmaceutical companies. However, they lack Fc fragments, making Fc-based detection and purification methods inapplicable. Additionally, pharmaceutical companies avoid adding tag proteins like Flag and His to scFv or VHH to prevent potential risks, meaning tag-based strategies are also unsuitable. For scFv and VHH without tags, current detection and purification methods face several challenges:

- Universal detection methods for scFv, such as Anti-Fab antibodies and Protein L, are not specifically developed for scFv, leading to compatibility issues, high background values, and low detection rates.
- No efficient universal detection and purification reagent exists for VHH.
- Idiotypic detection tools like anti-idiotypic antibodies and antigens are impractical for early-stage screening due to their long development cycles, high costs, and low applicability.

How GenScript Supports scFv and VHH Research with Advanced Solutions?

To support scFv and VHH drug research, GenScript offers a comprehensive solution with tools and reagents for every research stage. This includes antibody discovery, purification, functional identification, lentiviral transduction titer determination, CAR-T cell characterization and isolation, and pharmacokinetic detection. Our goal is to provide complete support for researchers to ensure success in scFv and VHH research.

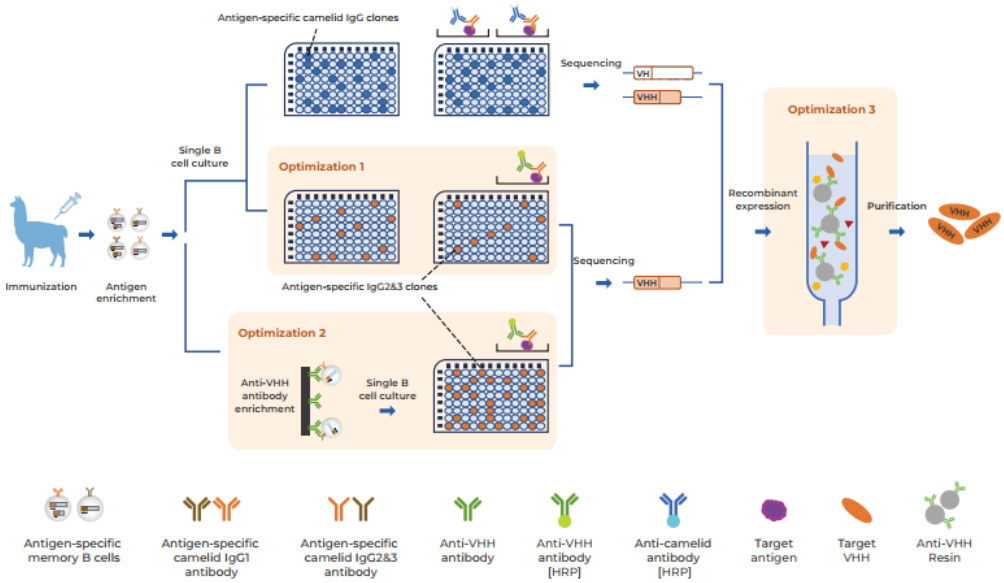


Application 1 - Antibody Discovery

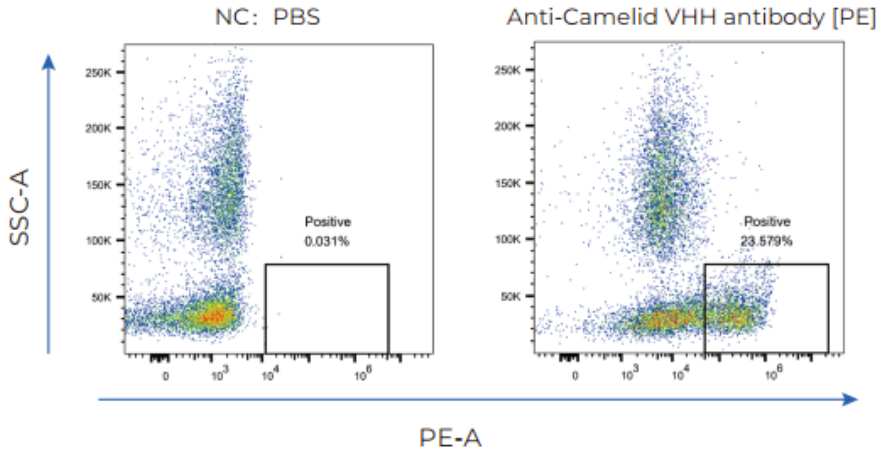
scFv and VHH antibody discovery is mainly achieved through phage display technology and B cell cloning technology. During the VHH antibody discovery process, the presence of traditional double-chain antibodies in camelid animals can interfere with VHH antibody screening, thereby reducing the efficiency of VHH antibody discovery. However, Anti-VHH antibodies can specifically enrich VHH+ cells and detect the expression of VHH antibodies, thus effectively aiding in the discovery of VHH antibodies.

Case Study 1: Optimization of B cell cloning technology in camelids

As non-VHH sequences can interfere with the screening of VHH antibodies using B cell cloning technology, additional plating and sequencing are usually applied to address this challenge. However, anti-VHH antibodies can enhance this process by enriching VHH+ B cells, which only secrete VHH antibodies, reducing the workload and cost, and improving efficiency. These antibodies can also be used during positive clone screening to ensure the clones are of the VHH subtype.



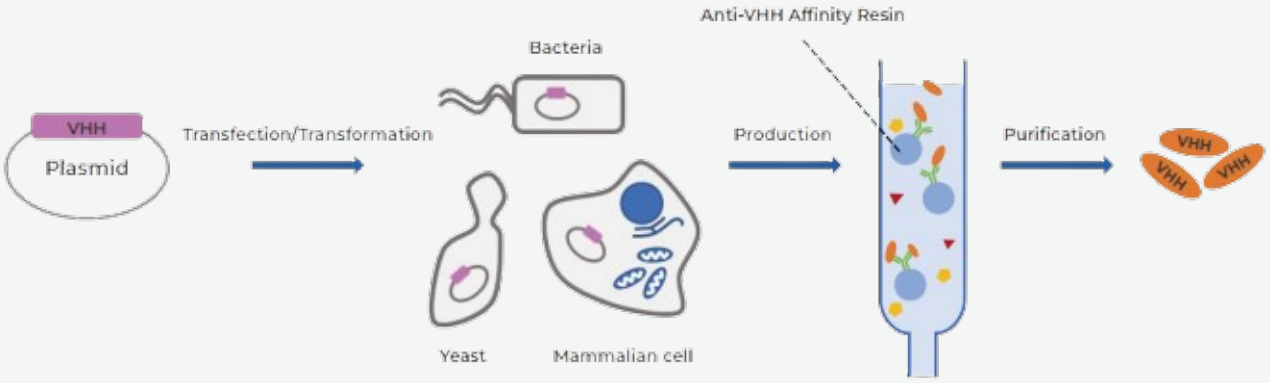
Application Data - Sorting PBMCs expressing VHH



FACS sorting of VHH+ PBMCs from non-immunized camels using 2 µg MonoRab™ Rabbit Anti-Camelid VHH Cocktail [PE] (GenScript A02018) shows approximately 23.579% VHH+ PBMCs, confirming A02018's high specificity for VHH+ PBMCs.

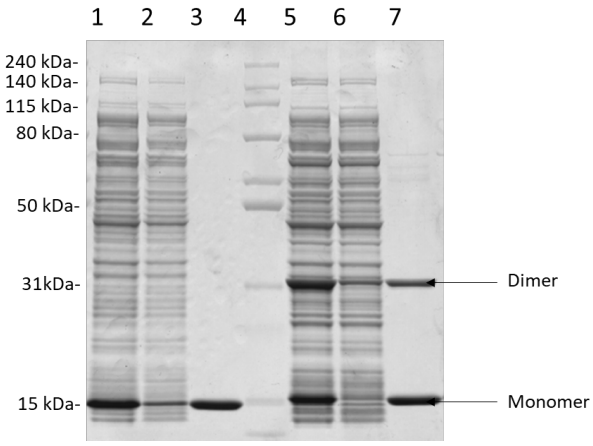
Application 2 - High-Specificity Antibody Purification

VHH purification is a necessary when studying VHH performance. While affinity chromatography with tags such as Fc or His is common, these tags pose potential risks for VHH drug development. Additionally, the humanization of VHH drugs, often required for human use to reduce immune reactions and improve safety, adds challenges to purification. Using purification media developed from Anti-VHH antibodies can efficiently purify camelid and humanized VHH from various expression systems without relying on tags, making it ideal for preclinical VHH drug research.



Purification Workflow of VHH Antibody

Case Study 1 - High-Specificity Purification of Camelid VHH Antibodies



Lane 1: Camelid VHH#1 whole cell lysate

Lane 2: VHH#1 flow through

Lane 3: VHH#1 eluate with L00905

Lane 4: Broad Multi Color Pre-Stained Protein Standard (GenScript, M00624)

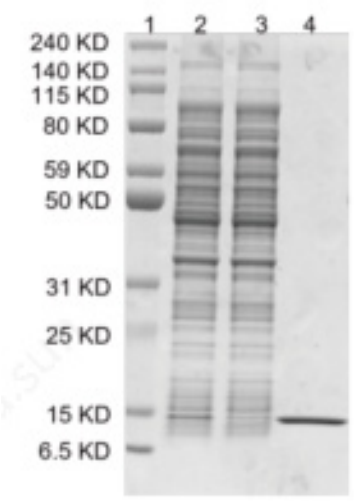
Lane 5: Camelid VHH#2 whole cell lysate (with dimer)

Lane 6: VHH#2 flow through

Lane 7: VHH#2 eluate with L00905

Purification of camelid VHH#1 and VHH#2 from E. coli lysate using MonoRab™ Anti-Camelid VHH Affinity Resin (GenScript, L00905) specifically yielded both monomeric and dimeric forms of VHH.

Case Study 2 - High-Specificity Purification of Humanized VHH Antibodies



Lane 1: Broad Multi Color Pre-Stained Protein Standard (GenScript, M00624)

Lane 2: Whole cell lysate

Lane 3: Flow through

Lane 4: Eluate of target humanized VHH single domain antibody-1

Purification of humanized VHH#1 from E. coli lysate using MonoRab™ Anti-Humanized VHH Affinity Resin FF (GenScript, L00951) demonstrated high specificity for humanized VHH.

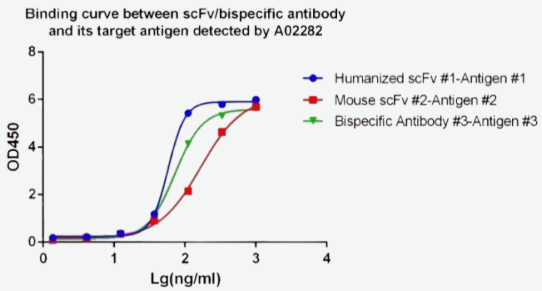
Application 3 – Antibody Functional Characterization

The antibody binding capacity of scFv or VHH to target antigens is crucial in drug development and screening. GenScript's anti-scFv and anti-VHH antibodies enable tag-free detection, speeding up the development of scFv or VHH drugs.

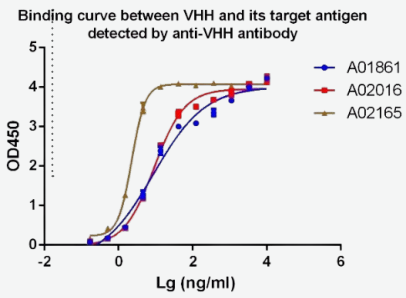
Case 1: Detection of scFv or VHH Binding to Target Antigen

The figure illustrates the use of Anti-scFv antibodies to detect the binding capacity of humanized scFv, mouse scFv, and bispecific antibodies to their target proteins (Figure A). Anti-VHH antibodies are used to assess the binding ability of VHH to its target protein (Figure B).

A



B



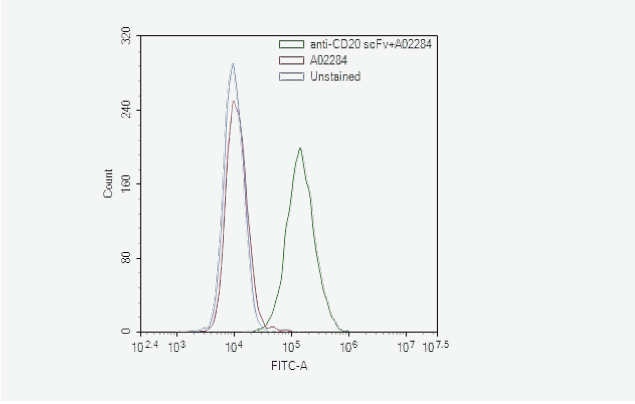
- Using Anti-scFv Antibody to detect the binding ability of scFv or scFv-based bispecific antibodies to their target antigens
  - Humanized scFv#1: anti-VEGF scFv; antigen#1: VEGF
  - Mouse scFv#2: anti-BCMA scFv; antigen#2: BCMA
  - Bispecific Antibody#3: anti-CD19×anti-CD3 (BiTE); antigen#3: CD3
- Using Anti-VHH Antibody to detect the binding ability of VHH to its target antigen.
  - Anti-VHH A02016: Detects anti-HIV p24 VHH binding to its target antigen HIV p24.

Case 2: Detection of scFv Binding to Target Cells

In developing scFv or scFv-based bispecific antibody drugs, assessing binding to cell surface proteins is crucial. Flow cytometry shows that Anti-scFv antibodies provide high specificity signals for cells binding scFv and low non-specific signals for cells without scFv, making them useful for detecting scFv binding to target cells.

Using MonoRab™ Rabbit Anti-scFv Cocktail [FITC] (GenScript, A02284) to analyze anti-CD20 antibodies' binding to Raji cells:

- Blue solid line: unstained cells
- Red solid line: cells with only A02284
- Green solid line: cells with both anti-CD20 scFv and A02284

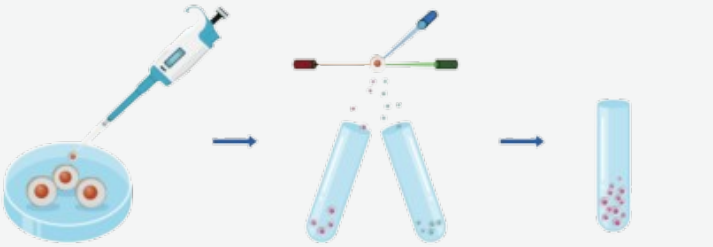


Application 4 – Virus Transduction Titer Determination

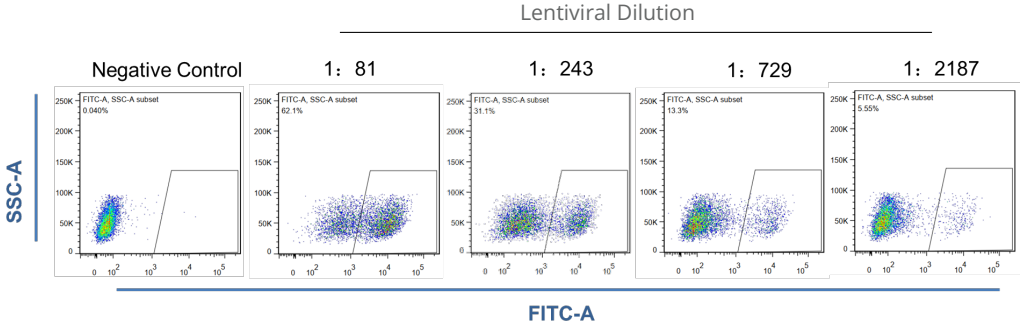
The most common application of scFv and VHH is in cell therapy, where they serve as the antigen recognition domain of CARs, enabling T lymphocytes to specifically recognize and target cell surface antigens, thereby exerting cell-specific cytotoxicity. Lentiviruses are crucial in CAR-T cell therapy, and determining their titer is essential for assessing the quality and functionality of the lentiviral vectors. Since lentiviral vectors cannot replicate, conventional cytopathic effect assays are not suitable for measuring viral titers. Instead, the titer is typically determined by evaluating the vector's ability to transduce sensitive cell lines or primary cells. This involves measuring the CAR expression positivity rate or CAR gene copy number in the transduced cells to calculate the transduction titer (TU/ml).

Case 1: Using Anti-scFv Antibodies for Lentivirus Transduction Titer Determination

Dilute the lentivirus carrying the CAR gene in a gradient and use it to infect the sensitive HEK-293 cell line to obtain CAR-expressing HEK-293 cells, where the CAR's antigen recognition domain is scFv.



Then, use FITC-labeled anti-scFv antibody (GenScript, A02284) to detect the CAR positivity rate (i.e., the percentage of fluorescent cells among the total cells) by flow cytometry. Calculate the viral transduction titer based on the virus dilution factor and the number of positive infected cells. The results show that cells without CAR and cells with CAR can be well differentiated, indicating that the anti-scFv antibody can effectively detect lentivirus transduction titer.



Formula: Viral titer = (CAR-positive cell proportion x Total cell number x Virus dilution factor) / Virus volume. The lentivirus transduction titer was calculated to be $2.19x10^8$ TU/ml.

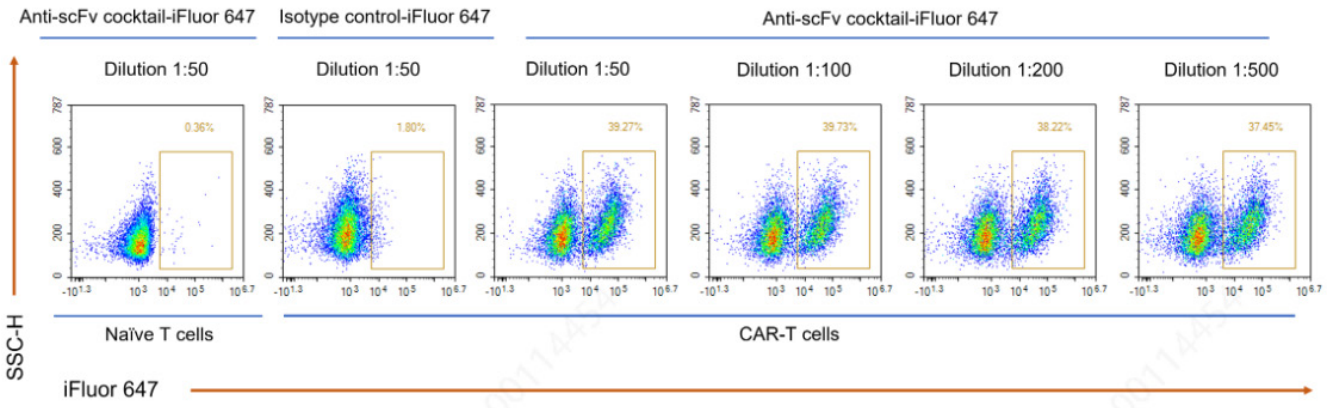
Application 5 – CAR-T Cell Characterization

In cell therapy, monitoring the expression level of scFv or VHH on the surface of cells is crucial for assessing CAR-T cell positivity. This detection is critical for several reasons. In June 2018, the China Food and Drug Administration issued guidelines titled "CAR-T Cell Therapy Product Quality Control Testing and Non-Clinical Research Considerations," highlighting the positivity rate of CAR-T cells as a key quality control indicator for cell products. The effectiveness and safety of CAR-T therapy rely on the presence of CAR-positive T cells, the components responsible for tumor cell elimination in vivo. Therefore, evaluating the CAR positivity rate is fundamental during both preclinical and clinical stages.

Beyond production quality control, clinical trials and post-market surveillance require assessing various aspects of cellular activity, such as patient remission time, CAR-T cell proliferation, and survival in the body. These evaluations depend on the quantity and strength of effective CAR structures in vivo. Therefore, detecting scFv or VHH on CARs is essential from preclinical research through post-market monitoring.

Case 1: Characterization of scFv-Based CAR-T Cells

GenScript's Anti-scFv antibody shows exceptional specificity for scFv-based CAR-T cells. Experimental results indicate that Anti-scFv antibodies, across various dilution ratios, can accurately detect CAR-T cell positivity rates. Furthermore, this antibody exhibits minimal non-specific binding to unedited T cells, making it ideal for CAR-T cell characterization.



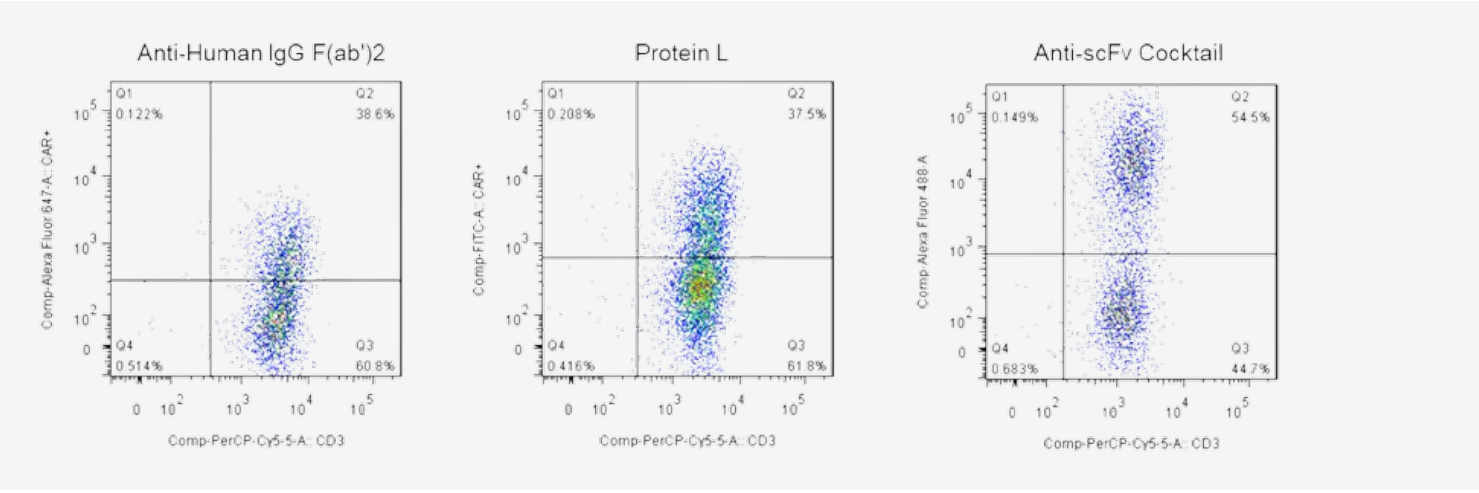
Characterization of scFv-Based CAR-T Cells

Various dilution ratios (1:50 to 1:500) of MonoRab™ Rabbit Anti-scFv Cocktail [iFluor 647] (GenScript, A02288) were used to detect CAR-T cells. MonoRab™ Rabbit IgG Control [iFluor 647] (Whole Molecule), mAb (GenScript, A02026), was used for staining CAR-T cells, with A02288 employed to stain naïve T cells as a negative control.

Case 2: Comparison of Characterization Tools for scFv-Based CAR-T Cells

Currently, various tools are available for CAR-T cell characterization, such as Protein L, which targets κ light chains, and anti-Fab antibodies, which target Fab fragments. However, these methods are generally designed for broad scFv detection rather than specifically targeting scFv.

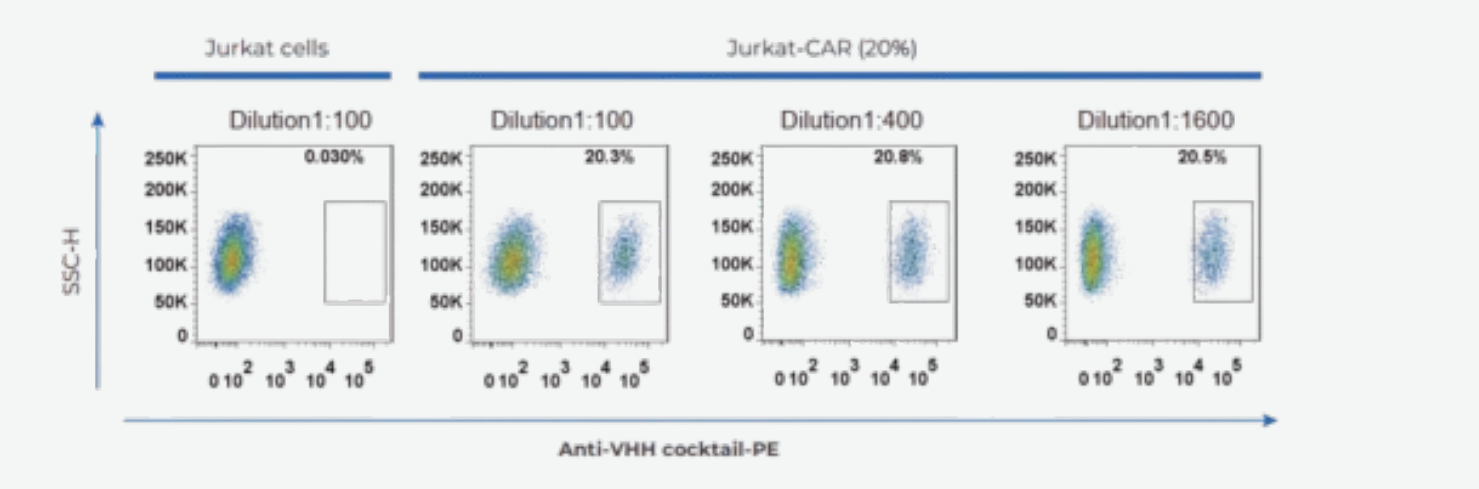
GenScript's MonoRab™ Rabbit Anti-scFv Antibody is specifically tailored for scFv, offering broader applications in scFv detection and CAR-T cell characterization. Flow cytometry results demonstrate that the Anti-scFv antibody provides superior cell gating and higher detection rates compared to Protein L and anti-Fab antibodies.



The same concentration of MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282), FITC-Labeled Recombinant Protein L, and Alexa Fluor® 647 AffiniPure Goat Anti-Human IgG, F(ab')2 was used to detect identical CAR-T cells. The fluorescence signal for the Anti-scFv antibody was provided by Alexa Fluor® 488-Labeled Goat Anti-Rabbit IgG.

Case 3: Characterization of VHH-Based CAR-T Cells

GenScript's Anti-VHH antibody demonstrates exceptionally high specificity for CAR-T cells with an extracellular VHH domain. Antibodies at various dilution ratios can accurately detect the number of VHH CAR-T cells in a sample.



FACS analysis of different dilution ratios of MonoRab™ Rabbit Anti-Camelid VHH Cocktail [PE] (GenScript, A02018) was conducted to assess binding activity to Jurkat cells, including 20% VHH-based Jurkat-CAR cells. The results indicate that A02018 specifically binds to VHH-based Jurkat-CAR cells, making it suitable for confirming and detecting CAR-T cells.

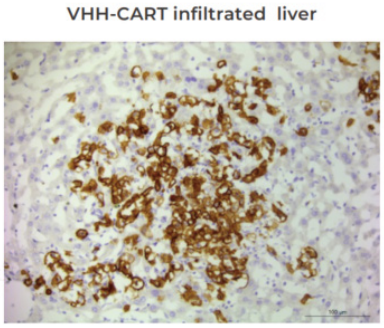
Case 4: Localization of VHH-Based CAR-T Cells

Although CAR-T cell therapy has been successful in treating certain types of cancer, such as advanced leukemia, its efficacy in treating solid tumors remains limited. The primary challenge is that CAR-T cells cannot penetrate the physical barriers of solid tumors. To enhance the effectiveness of CAR-T cells, it is crucial to assess their distribution within tissues. Additionally, understanding the distribution of CAR-T cells provides essential information for predicting potential adverse reactions.

GenScript's MonoRab™ Anti-Camelid VHH Cocktail specifically binds to VHH-transduced CAR-T cells infiltrating the spleen of NCG mice and the liver of PDX mice. Both immunofluorescence and immunohistochemistry analyses can be employed to detect the distribution of CAR-T cells in tissues.

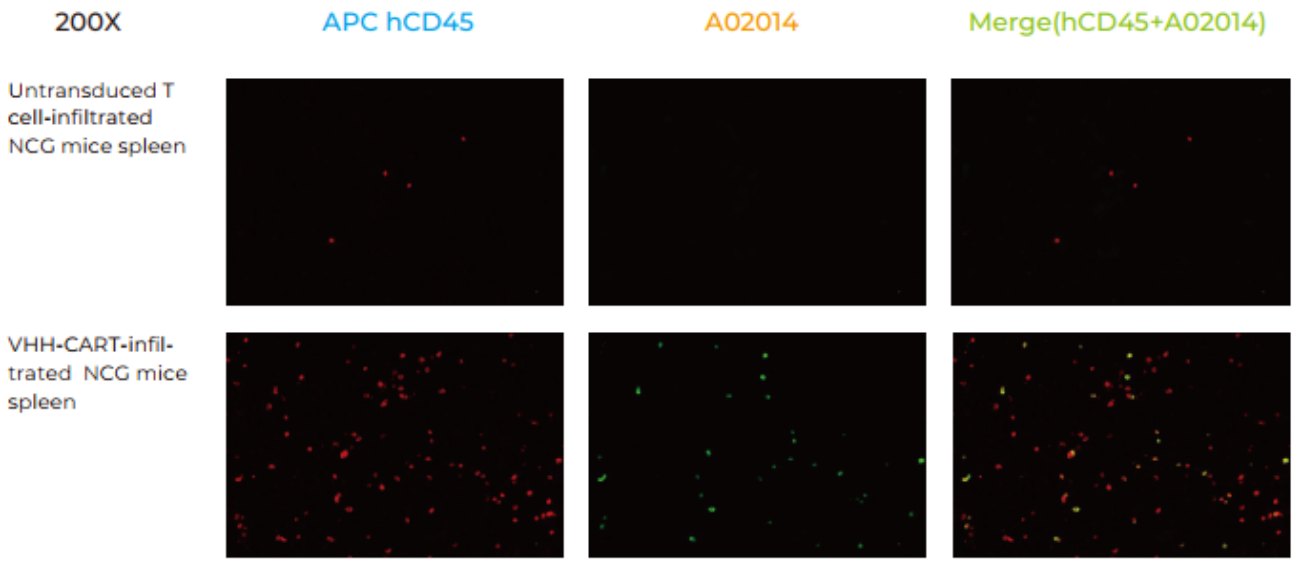
Immunohistochemical Analysis

VHH-transduced CAR-T cells infiltrating the liver tissue of PDX mice were analyzed. The primary antibody used was the MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) at 1 μg/mL. The secondary antibody was a biotin-labeled Anti-Rabbit IgG (H+L) at 2.5 μg/mL. Signal amplification was achieved using an ABC (Avidin-Biotin Complex) reagent kit, with counterstaining performed using hematoxylin. Results showed positive signals on the cell membrane or cytoplasm of VHH-transduced CAR-T cells infiltrating the liver tissue.



Immunofluorescence Analysis

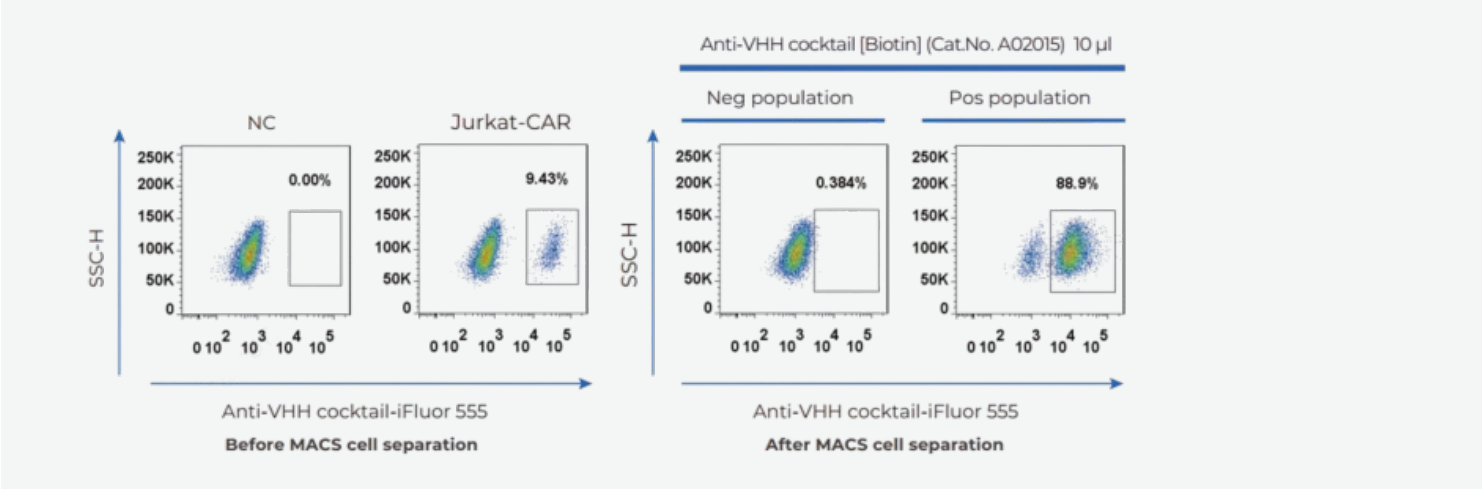
VHH-transduced CAR-T cells infiltrating the spleen tissue of NCG mice were examined. The primary antibody used was the MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) at 0.5 μg/mL. The secondary antibody was Anti-Rabbit IgG (H+L) [Alexa Fluor 488] (green) at 4 μg/mL. Additionally, a 0.5 μg/mL APC-labeled anti-hCD45 monoclonal antibody was used to detect all injected T cells (red). Confocal images showed positive signals in the cytoplasm of VHH-transduced CAR-T cells infiltrating the spleen tissue of NCG mice, while non-transduced infiltrating T cells exhibited negative signals.



Application 6 – CAR-T Cell Isolation

In cell therapy, CAR-positive T cells are the key components that exert tumor-killing effects. The proportion of CAR-T cells obtained through genetic modification does not always meet the desired levels, particularly in non-viral vector infections where CAR positivity rates can be lower. Therefore, it is necessary to enrich CAR-T cells and expand a high percentage of CAR-positive T cells to achieve the best therapeutic effect.

Case 1: Enrichment of VHH-Based CAR-T Cells



FACS analysis using MonoRab™ Rabbit Anti-Camelid VHH Cocktail [Biotin] (GenScript, A02015) and anti-Biotin magnetic beads was conducted to evaluate the sorting efficiency of VHH-based CAR-T cells via MACS technology.

Naïve T cells served as a negative control, and the detection antibody for FACS analysis was MonoRab™ Rabbit Anti-Camelid VHH Cocktail [iFluor 555] (GenScript, A02020). The results, shown in the figure, demonstrate that after MACS cell separation, the percentage of positive CAR-T cells increased from 10% to 90%, indicating that A02015 is an ideal tool for MACS separation of CAR-T cells.

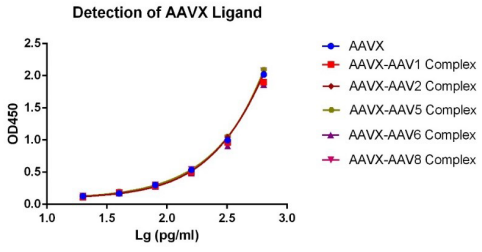
Application 7 – Residual Affinity Ligand Detection

Affinity chromatography is a liquid-phase chromatography technique that utilizes affinity adsorption media with conjugated affinity ligands as the stationary phase to selectively adsorb target products, achieving their separation and purification. VHH, a next-generation purification ligand, offers numerous advantages including high specificity, high affinity, structural stability, and high capacity. Thermo's CaptureSelect™ series products, which use VHH as the affinity ligand, are widely employed for the purification of antibodies, proteins, enzymes, AAVs, and more.

For products intended for human therapy, it is crucial to detect residual affinity ligands in the purified product to mitigate risks associated with ligand leaching. GenScript, the only company offering VHH monoclonal antibodies, has developed the VHH Affinity Ligand ELISA Kit using high-performance anti-VHH antibodies. This kit provides high sensitivity in detecting various VHH ligands while minimizing inter-batch variation.

Case 1: Detection of AAVX Affinity Ligand Residue

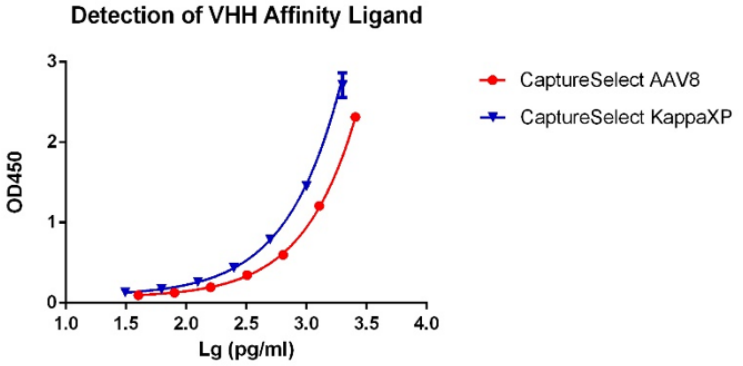
Affinity chromatography is a key method for AAV purification. The POROS™ CaptureSelect™ AAVX Affinity Resin (Thermo, A36739) is widely used due to its ability to bind multiple AAV serotypes, including AAV1 through AAV8 and AAVrh10. Since AAVs are major vectors for gene therapy and will be directly used in human treatments, detecting residual AAVX affinity ligand in AAV samples is crucial. GenScript's VHH Affinity Ligand ELISA Kit, which includes AAVX standards, offers a sensitivity of up to 20 pg/mL for AAVX. When AAVX forms complexes with AAV, the coefficient of variation (CV) for each concentration point is < 10% compared to individual AAVX detection, making this kit suitable for detecting residual CaptureSelect™ AAVX affinity ligands.

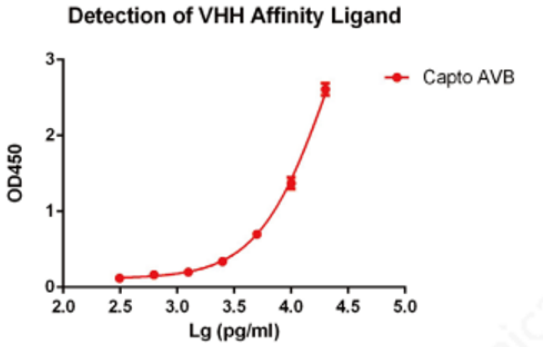


After adding AAV1, AAV2, AAV5, AAV6, and AAV8 to AAVX to form complexes, the consistency of the standard curves for AAVX and AAVX-AAV complexes was detected using the VHH Affinity Ligand ELISA kit (GenScript, L01033).

Case 2: Detection of Residues of AAV8, KappaXP, and AVB Affinity Ligands

In addition to detecting CaptureSelect™ AAVX residues, the VHH Affinity Ligand ELISA Kit can also identify residuals of other affinity ligands, such as CaptureSelect™ AAV8, CaptureSelect™ KappaXP, and Capto AVB, with sensitivities of 40 pg/mL, 31.25 pg/mL, and 312.5 pg/mL, respectively. Thus, the VHH Affinity Ligand ELISA Kit is an ideal tool for detecting VHH affinity ligand residues. The detection of standard curves for CaptureSelect™ AAV8, CaptureSelect™ KappaXP, and Capto AVB using the VHH Affinity Ligand ELISA Kit is illustrated.





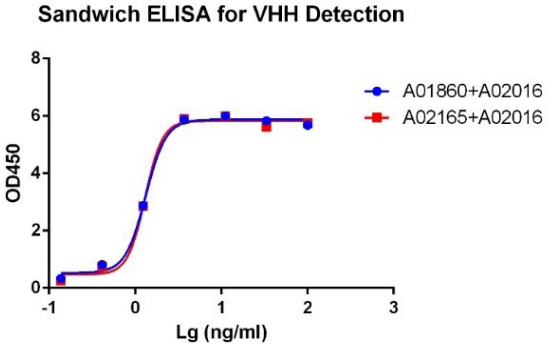
The above figures illustrate the detection of standard curves for CaptureSelect™ AAV8, CaptureSelect™ KappaXP, and Capto AVB using the VHH Affinity Ligand ELISA Kit.

Application 8 – Drug Quantitative Analysis

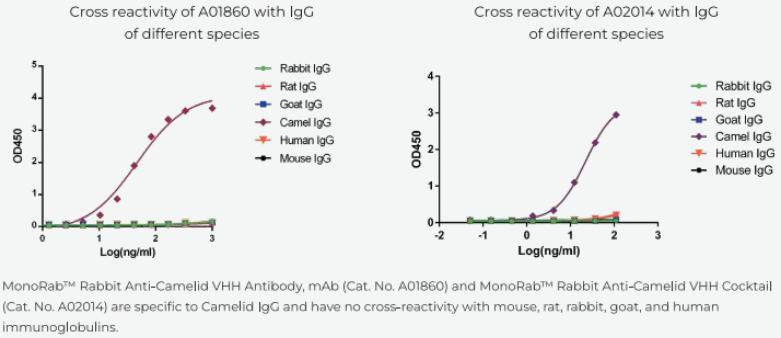
In some early-stage preclinical pharmacokinetic (PK) studies, anti-human IgG Fc antibodies are often used to detect non-human species PK samples due to considerations of time, cost, and the difficulty in obtaining anti-unique antibodies quickly. However, this method is unsuitable for nanobody PK detection because nanobodies lack Fc fragments. GenScript's Anti-VHH antibodies have been validated to show no cross-reactivity with IgG from various species, including humans, rabbits, mice, and sheep. Therefore, these antibodies are not only suitable for early-stage clinical screening of nanobody PK studies but can also serve as a partial replacement for anti-unique antibodies in detecting nanobody drug metabolism in humans.

Case 1. Sandwich ELISA for Detecting VHH

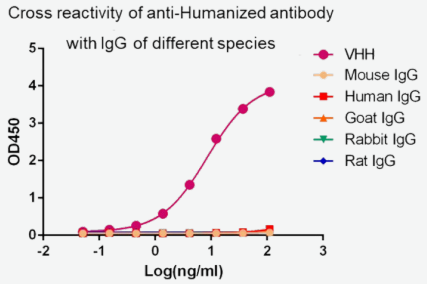
Sandwich ELISA standard curve for VHH: Using MonoRab™ Rabbit Anti-Camelid VHH (GenScript, A01860) or MonoRab™ Rabbit Anti-Humanized VHH (GenScript, A02165) as the capture antibody, and MonoRab™ Rabbit Anti-Camelid VHH Cocktail [HRP] (GenScript, A02016) as the detection antibody, with sensitivity reaching up to 137 pg/mL.



Case 2. Anti-VHH Antibody Specificity



MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) are specific to Camelid IgG and have no cross-reactivity with mouse, rat, rabbit, goat, and human immunoglobulins.



MonoRab™ Rabbit Anti-Camelid VHH (GenScript, A01860) specifically binds to camelid VHH; MonoRab™ Rabbit Anti-Humanized VHH Antibody, mAb (GenScript, A02165) specifically binds to humanized VHH; and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) binds to both camelid and humanized VHH. These three Anti-VHH antibodies do not cross-react with IgG from mouse, rat, rabbit, goat, or human, making them ideal for quantitative drug analysis across various matrices.

Product Selection Guide

GenScript's Anti-VHH antibody products include two sets of highly specific monoclonal antibodies, along with a mixture designed for broad-spectrum VHH detection. The Anti-scFv antibody products feature a mixture of monoclonal antibodies for broad-spectrum scFv detection, as well as a set with minimal cross-reactivity to IgGs from different species. With a wide selection available, these products meet the detection needs for scFv and VHH across various species. Additionally, a range of conjugates and derivative products are offered to accommodate diverse experimental design requirements.

Product Type		Species Specificity	Uncon-jugated	Conjugated						
				HRP	Biotin	iFluor 488	iFluor 555	iFluor 647	PE	FITC
Anti-VHH antibody	Anti-Camelid VHH,mAb	Llama, Camel, Alpaca	A01860	A01861	A01995	A01862	A01863	A01994	A02227	A02227
	Anti-Humanized VHH, mAb	Humanized, Llama, Camel, Alpaca	A02165	A02167	A02166	A02168	A02169	A02170	A02171	A02227
	Anti-Camelid VHH, mAb Cocktail	Humanized, Llama, Camel, Alpaca	A02014	A02016	A02015	A02021	A02020	A02019	A02018	A02227
Anti-scFv antibody	Anti-scFv Antibody Cocktail	Humanized, Mouse	A02282	A02289	A02283	A02286	A02287	A02288	A02285	A02227
	Anti-scFv Antibody Cocktail (Min X)*	Humanized, Mouse		A02306	A02303	A02304		A02305	A02315	
	GS Linker Antibody							A02311	A02314	

Application								
Flow cytometry	✓		✓	✓	✓	✓	✓	✓
Western blot / Dot blot	✓	✓	✓					
ELISA	✓	✓	✓					
Immunohistochemistry	✓	✓	✓					
Immunofluorescence	✓		✓	✓	✓	✓	✓	✓
Cell sorting	✓		✓	✓	✓	✓	✓	✓

*Anti-scFv antibody Cocktail (Min X) has no cross-reactivity to human IgG, mouse IgG, goat IgG and rabbit IgG.

Anti-VHH and anti-scFv antibody derivatives

Product Number	Product Name
L00905	MonoRab™ Anti-Camelid VHH Affinity Resin
L00946	MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate (Clear, 8×12 strip)
L00951	MonoRab™ Anti-Humanized VHH Affinity Resin FF
L01008	MonoRab™ Rabbit Anti-VHH Microbeads
L01033*	VHH Affinity Ligand ELISA kit
L01034	MonoRab™ Anti-VHH Affinity Magnetic Beads

*The L01033 VHH Affinity Ligand ELISA kit already contains the CaptureSelect™ AAVX standard. If you want to detect Capto AVB CaptureSelect™ AAV8 or CaptureSelect™ KappaXP, you need to purchase the AVB Affinity Ligand Standard (GenScript, Z03794) AAV8 Affinity Ligand Standard (GenScript, Z03795) or KappaXP Affinity Ligand Standard(GenScript, Z03796) separately.

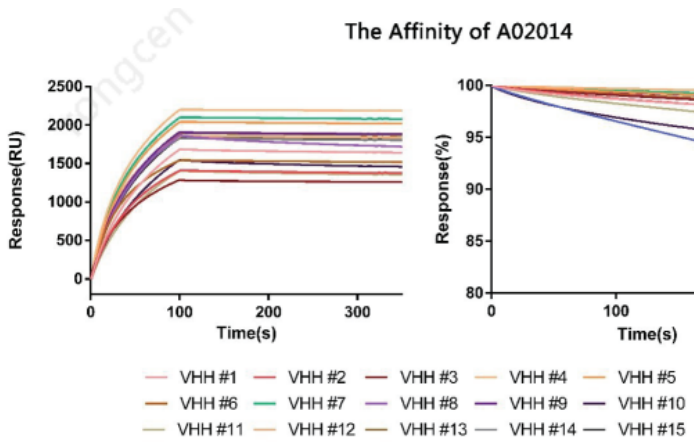
Other products

Product Type	Unconjugated	Conjugated						
		HRP	Biotin	iFluor 488	iFluor 555	iFluor 647	PE	FITC
Mouse Anti-Human IgG Fab Antibody (12H3C4A6), mAb		A01855						
protein L		M00098	M00097	M00921		M00922		M00920
Mouse Anti-Human IgG Fc Antibody (50B4A9), mAb	A02257	A01854						
Streptavidin	Z02043	M00091						
MonoRab™ Rabbit IgG Control (Whole Molecule), mAb	A02022			A02028	A02027	A02026	A02025	
Human Kappa Light Chain (2F1C1), mAb, Mouse	V06801							
Human Lambda Light Chain (2D54), mAb, Mouse	V06901							
MonoRab™ Biotin Antibody (53C8), mAb, Rabbit	V90201			A02028		A02249	A02250	

Features of Anti-VHH Antibody

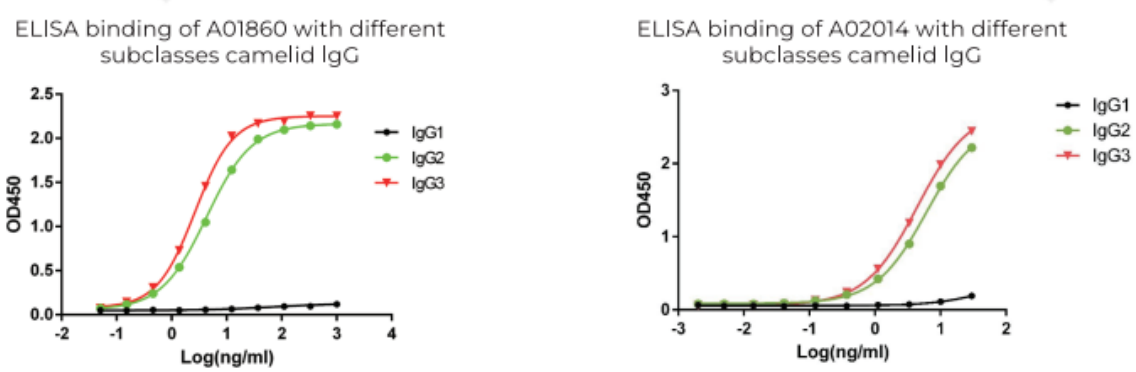
Antibody	Anti-Camelid VHH, mAb	Anti-Humanized VHH, mAb	Anti-Camelid VHH, mAb Cocktail
Products	A01860, A01861, A01995, A01862, A01863, A01994, A02226, A02227	A02165, A02167, A02166, A02168, A02169, A02170, A02171, A02172	A02014, A02016, A02015, A02021, A02020, A02019, A02018, A02017
Binding compatibility	★ ★	★ ★	★ ★ ★
Affinity to certain VHH	★ ★ ★	★ ★ ★	★ ★
Specificity to Humanized VHH	★	★ ★ ★	★ ★
Specificity to Camelid VHH	★ ★ ★	★	★ ★
VHH Discovery	★ ★	★	★ ★ ★
Detection and analysis of certain VHH	★ ★ ★	★ ★ ★	★ ★

MonoRab™ technology ensures high affinity with a Kd ≈ 10^{-11} M



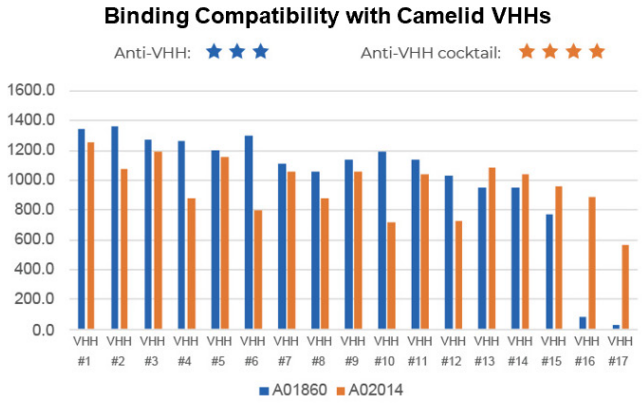
The affinity of MonoRab™ Rabbit Anti-Camelid Cocktail (Cat. No. A02014) with 15 random VHHs is measured by Biacore. The cocktail antibody shows comprehensive binding activity and high affinity with all of the 15 VHHs. Instead of using anti-VHH polyclonal antibodies which have a potential lot-to-lot consistency issue, anti-VHH cocktail antibody is the best choice for VHH direct detection. It is also not necessary to add a tag to VHH for detection.

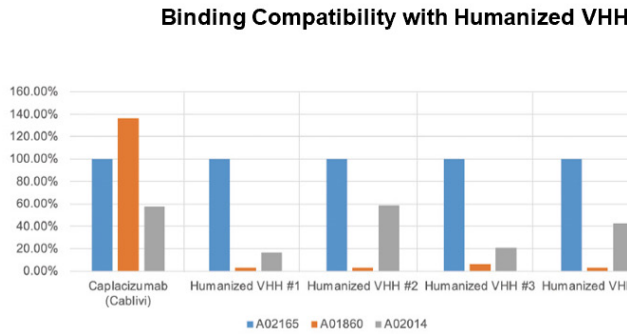
Specific to Camelid IgG2 & IgG3



MonoRab™ Rabbit Anti-Camelid VHH (GenScript, A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) specifically bind to the variable region of camelid heavy-chain antibodies (IgG2 & 3) and do not recognize the IgG1 subtype, which follows a conventional antibody structure.

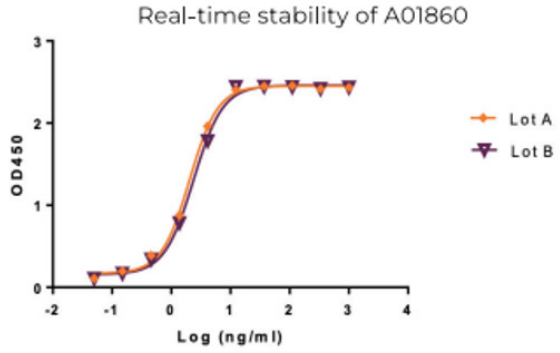
Broad Binding Compatibility



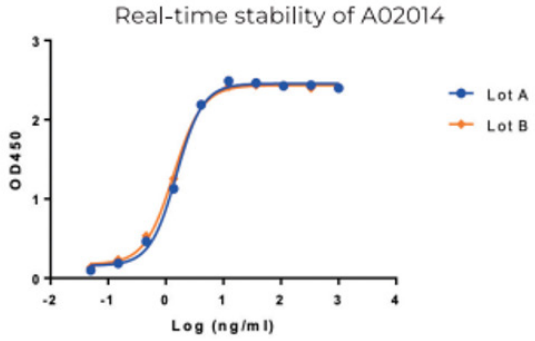


MonoRab™ Rabbit Anti-Camelid VHH Antibody (GenScript, A01860) offers broad binding compatibility of camelid VHH, MonoRab™ Rabbit Anti-Humanized VHH Antibody (GenScript, A02165) is optimized for humanized VHH, and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) effectively recognizes both camelid and humanized VHH.

High Stability



· Lot A was produced in 2018
· Lot B was produced in 2020

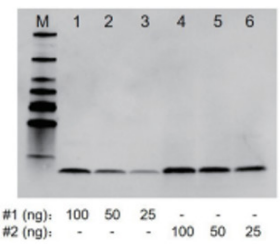


· Lot A was produced in 2019
· Lot B was produced in 2021

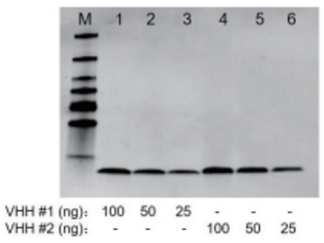
MonoRab™ Rabbit Anti-Camelid VHH (GenScript, A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) remain stable for at least two years.

High Sensitivity

Western Blot of A01860 with VHHs



Western Blot of A02014 with VHHs

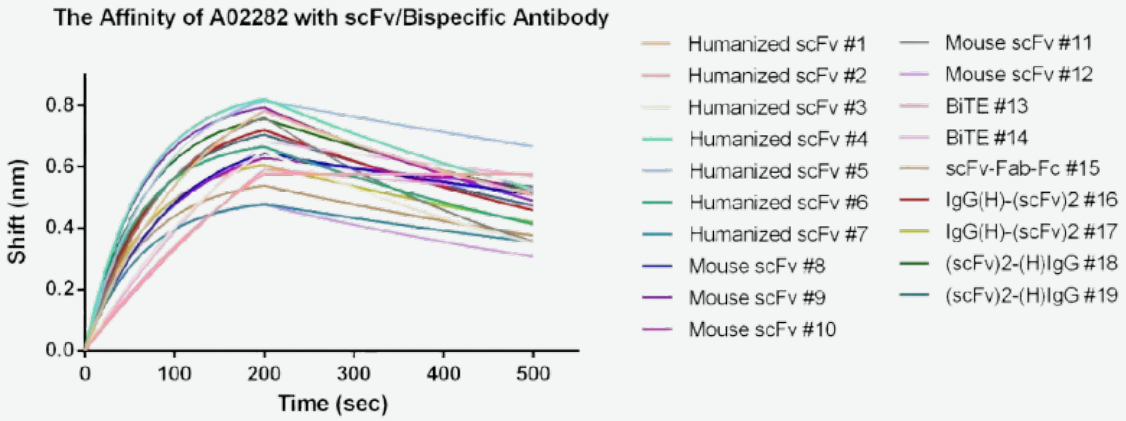


Lane 1: VHH #1 100 ng
Lane 2: VHH #1 50 ng
Lane 3: VHH #1 25 ng
Lane 4: VHH #2 100 ng
Lane 5: VHH #2 50 ng
Lane 6: VHH #2 25 ng

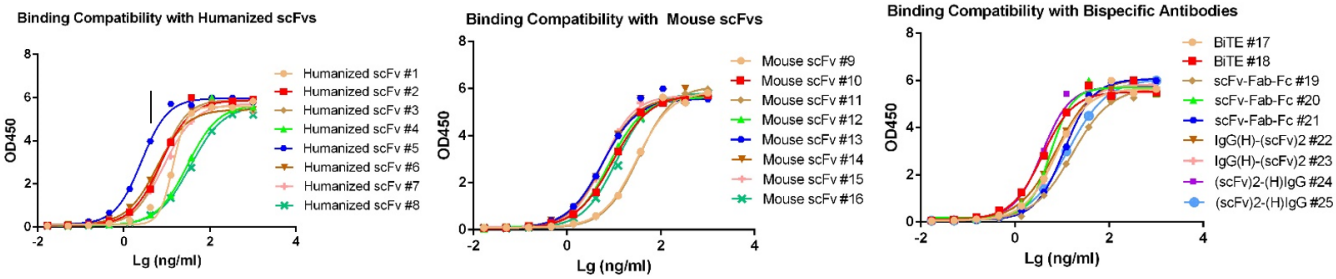
Western blot results show that both MonoRab™ Rabbit Anti-Camelid VHH (GenScript, A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) exhibit high sensitivity for detecting VHH.

Features of Anti-scFv Antibody

MonoRab™ Technology Ensures High Affinity

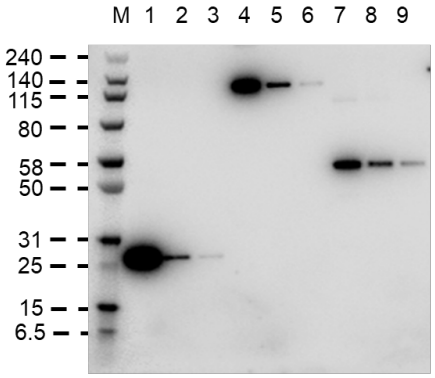


Biolayer interferometry (BLI) results show that the anti-scFv antibody exhibits high affinity for scFvs from various sources, regardless of the order of the variable regions (VH-VL or VL-VH), as well as for different forms of scFv-based bispecific antibodies.



ELISA results indicate that MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) specifically recognizes scFvs with different sources and variable region orders (VH-VL or VL-VH), as well as various scFv-based bispecific antibodies like BiTE, IgG(H)-(scFv)2, and scFv-Fab-Fc.

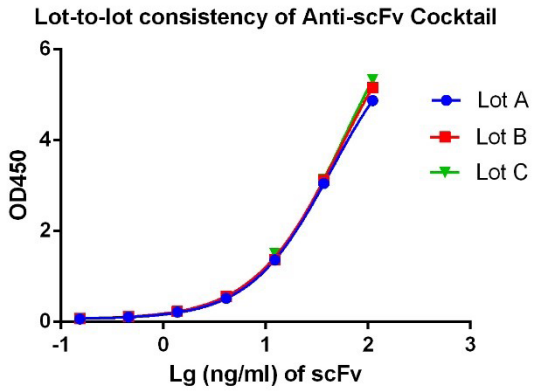
High Sensitivity



M: Protein Marker (GenScript, M00624)
Lane 1: Humanized scFv 50ng
Lane 2: Humanized scFv 25ng
Lane 3: Humanized scFv 10ng
Lane 4: Mouse scFv-Fc 50ng
Lane 5: Mouse scFv-Fc 25ng
Lane 6: Mouse scFv-Fc 10ng
Lane 7: BiTE 50ng
Lane 8: BiTE 25ng
Lane 9: BiTE 10ng

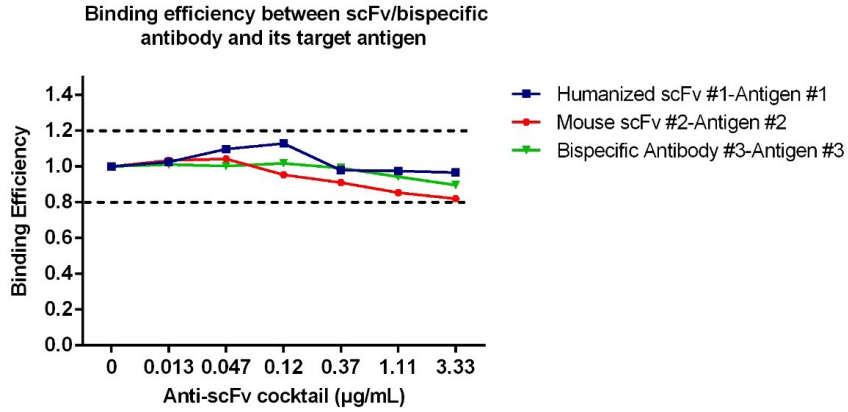
Western blot results demonstrate that MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) exhibits high sensitivity for scFvs from various sources as well as for the bispecific antibody BiTE.

High Consistency



Three batches of MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) show consistent binding curves for scFv, with a coefficient of variation (CV) of less than 10% at all concentration points.

Non-blocking Binding



Competitive ELISA results show that the Anti-scFv antibody does not interfere with scFv's binding to its target antigen after binding to scFv.

References

1 Monnier P , Vigouroux R , Tassew N .In Vivo Applications of Single Chain Fv (Variable Domain) (scFv) Fragments[J]. Antibodies, 2013, 2(4):193-208.DOI:10.3390/antib2020193.

2 Van Audenhove I , Gettemans J .Nanobodies as Versatile Tools to Understand, Diagnose, Visualize and Treat Cancer[J].EBioMedicine, 2016, 8(C).DOI:10.1016/j.ebiom.2016.04.028.

3 Asaadi Y , Jouneghani F F , Janani S ,et al.A comprehensive comparison between camelid nanobodies and single chain variable fragments[J].Biomarker Research, 2021, 9(1):87-.DOI:10.1186/s40364-021-00332-6.

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Learn More about Anti-VHH Antibody

Learn More about Anti-scFv Antibody