GenScript
Make Research Easy

MonoRab™ Anti-VHH Antibodies

Unique Tools for Detecting Camelid/Humanized Single Domain Antibodies

CONTENTS

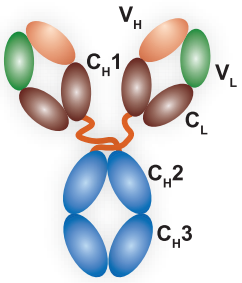
Introduction

Single domain antibody (VHH) possesses unique properties, such as small size, robust structure, high solubility, and accessibility to hidden and essential epitopes. Despite its small size (about 15 kDa), VHH is capable of binding an antigen with high specificity and affinity. Thus, VHH is a promising tool in therapeutics and immunodiagnostics applications, such as developing recombinant antibody drugs and CAR-T therapy.

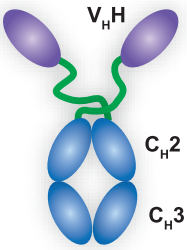
What is VHH?

Members of the Camelidae family including llamas, alpacas, and camels produce two different types of IgG antibodies. One type is the conventional IgG1 (MW~150 kDa) composed of two heavy chains and two light chains. The other type includes IgG2 and IgG3 (MW~90 kDa) which lack the light chains as well as one constant domain of the heavy chain (CH1 domain). Those antibodies are commonly called heavy chain antibodies (HCAbs). The variable domain of the HCAbs is referred to as single domain antibody, also called sdAb or VHH. VHH is considered the smallest antigen-binding entity.

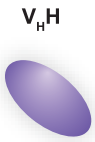
In therapeutic applications, camelid VHH needs to be humanized to reduce the risk of immunogenicity and anti-drug antibody (ADA) development in humans. Likewise, adding a tag (His or Fc) is not allowed since it may cause adverse immunological responses.



Conventional IgG
(Camelid Ig G1)



Camelid Ig G2 or
Ig G3



Single domain
antibody (sdAb)

What GenScript offers?

GenScript has developed a series of rabbit monoclonal (MonoRab™) anti-VHH antibodies to facilitate your research of VHHs. Our anti-VHH antibodies recognize conformational epitopes of the VHH domain of camelid IgG2 & IgG3 subclasses. It is not necessary to add a tag to VHH for detection anymore.

Applications

- VHH antibody screening or titer determination
- Selection of heavy chain antibody-expressing B cells in peripheral blood mononuclear cells (PBMC)
- Expression evaluation and sorting of CAR-T cells harboring a camelid or humanized single domain antibody

Product Selection Guide

GenScript's anti-VHH antibody portfolio includes two series of high specificity monoclonal antibodies and one series of broad detection monoclonal cocktail antibodies. The wide selection covers detection of both camelid and humanized VHHs. Moreover, various conjugates are available to meet your different requirements for assay development.

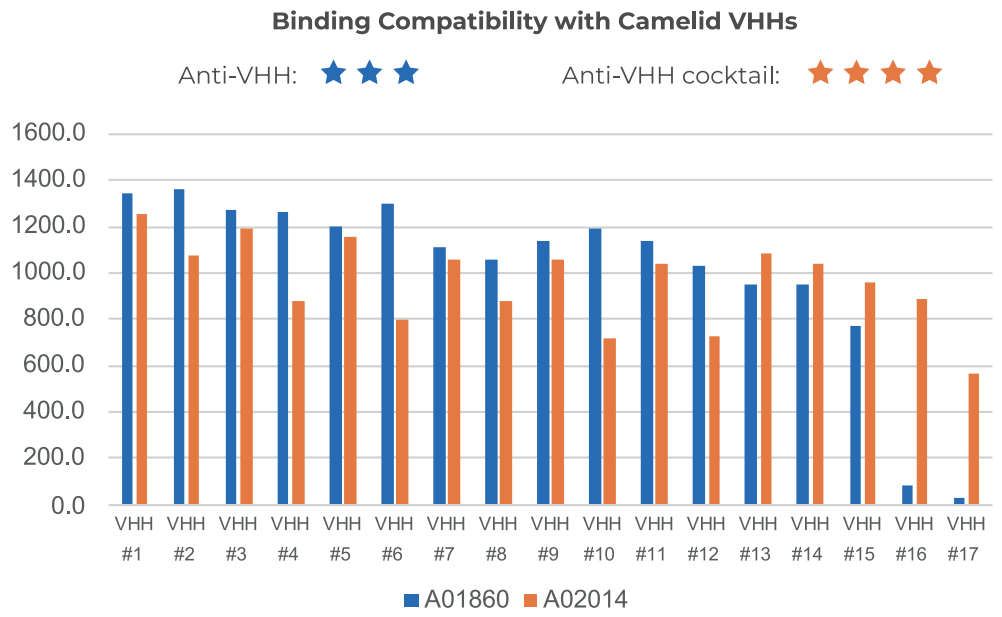
Anti-VHH antibody product list

Product Type	Species Specificity	Unconjugated	Conjugated						
			HRP	Biotin	iFluor 488	iFluor 555	iFluor 647	PE	FITC
Anti-Camelid VHH, mAb	Llama, Camel, Alpaca	**A01860**	A01861	A01995	A01862	A01863	A01994		
Anti-Humanized VHH, mAb	Humanized, Llama, Camel, Alpaca	**A02165**	A02167	A02166	A02168	A02169	A02170		
Anti-Camelid VHH, mAb Cocktail	Llama, Camel, Alpaca	**A02014**	A02016	A02015	A02021	A02020	A02019	A02018	A02017

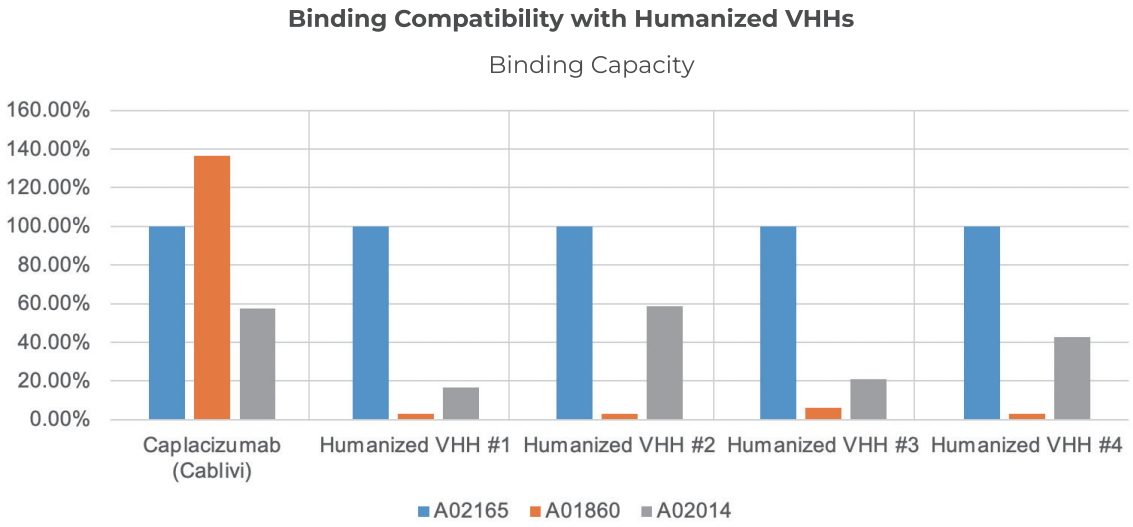
Anti-VHH antibody comparison overview

Clone	96A3F5	45H8	/
Antibody Type	mAb	mAb	mAb Cocktail
Products	A01860, A01861, A01995, A01862, A01863, A01994	A02165, A02167, A02166, A02168, A02169, A02170	A02014, A02016, A02015, A02021, A02020, A02019, A02018, A02017
Binding compatibility	★	★	★★
Affinity to single VHH	★★	★★	★
Specificity to Humanized VHH	★	★★	★
Specificity to Camelid VHH	★★	★	★

Comparison data



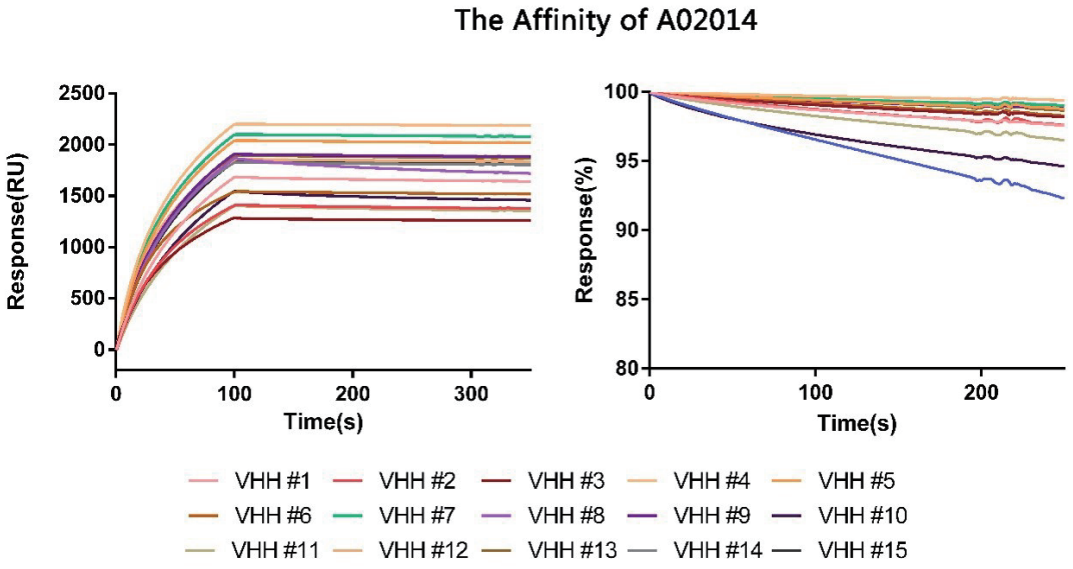
Binding compatibility comparison of MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) with camelid VHHs. The performance of the cocktail antibody (Cat. No. A02014) is better. It is ideal to be used for VHH antibody screening.



Binding compatibility comparison of MonoRab™ Rabbit Anti-Humanized VHH Antibody, mAb (Cat. No. A02165), MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860), and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) with humanized VHHs. The performance of A02165 is the best. It is ideal to be used for humanized VHH antibody detection.

Features

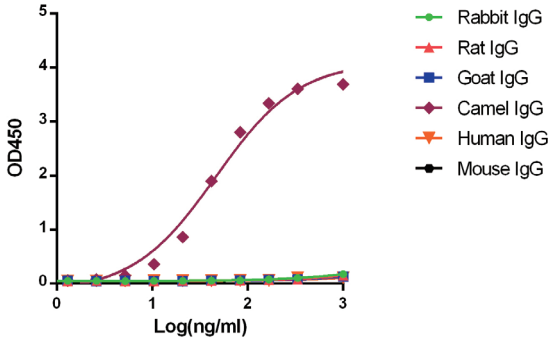
MonoRab™ technology guarantees high affinity: Kd ≈ 10^{-11} M



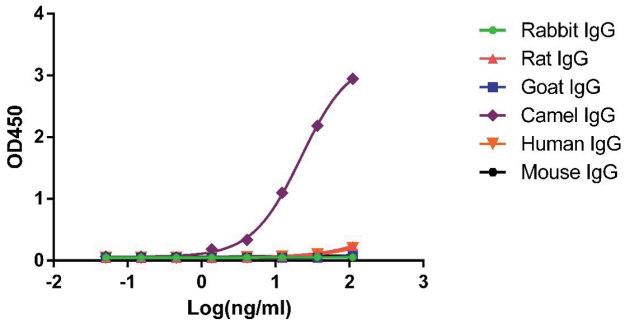
The affinity of MonoRab™ Rabbit Anti-Camelid Cocktail (Cat. No. A02014) with 15 random VHHs is measured by Biacore. The cocktail antibody shows comprehensive binding activity and high affinity with all of the 15 VHHs. Instead of using anti-VHH polyclonal antibodies which have a potential lot-to-lot consistency 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.

No cross-reactivity with other species

Cross reactivity of A01860 with IgG of different species



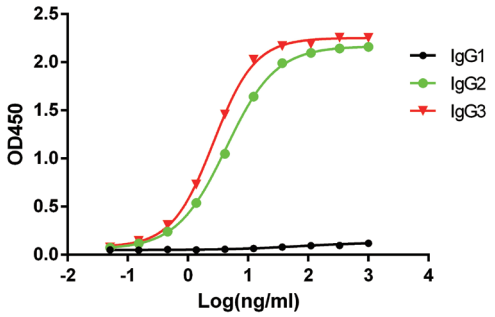
Cross reactivity of A02014 with IgG of different species



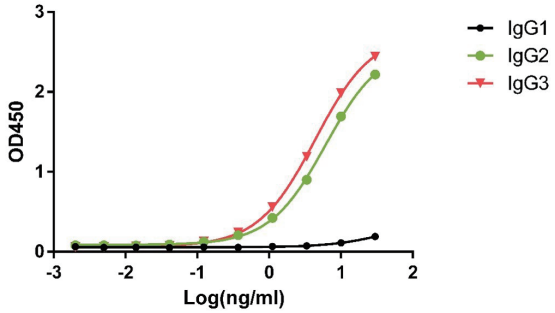
MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) are specific to Camelid IgG and have no cross-reactivity with mouse, rat, rabbit, goat, and human immunoglobulins.

Specific to camelid IgG2 & IgG3

ELISA binding of A01860 with different subclasses camelid IgG



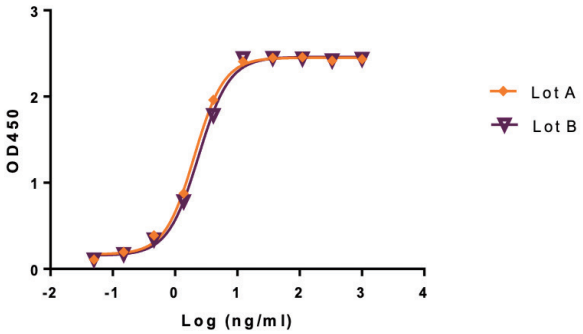
ELISA binding of A02014 with different subclasses camelid IgG



MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) are specific to the variable domain of camelid heavy chain antibodies (IgG2&3). It is ideal for isolation of heavy chain antibody expressing PBMC cells or B cells. Hence, specific VHH gene libraries can be generated for nanobody development.

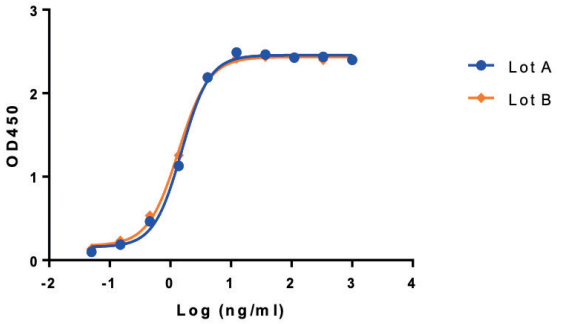
High stability

Real-time stability of A01860



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

Real-time stability of A02014



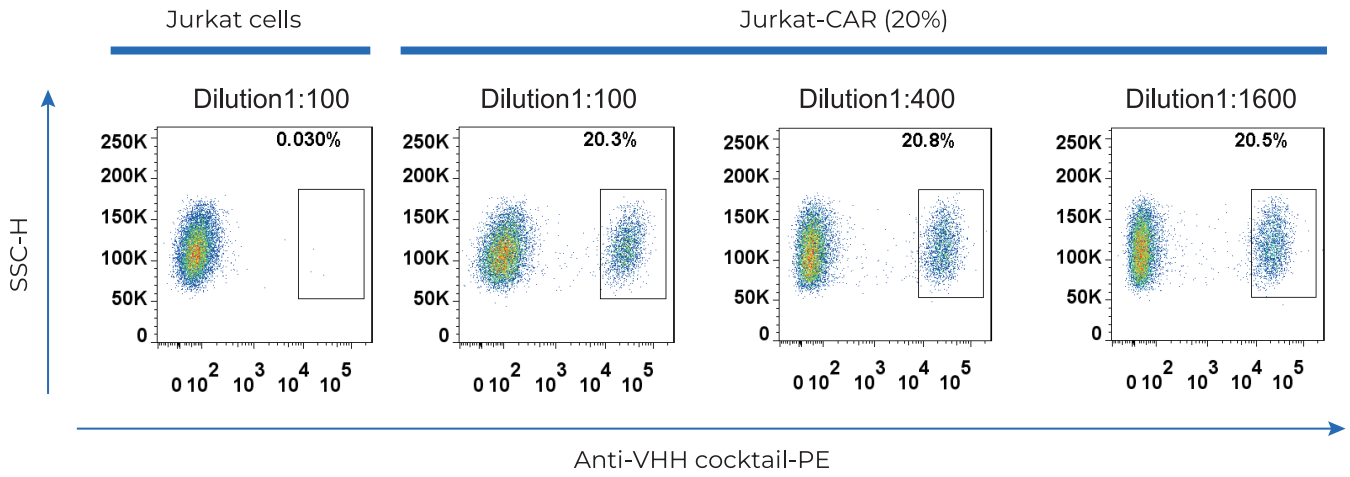
- Lot A was produced in 2019
- Lot B was produced in 2021

MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) can remain stable for at least 2 years.

Application Validations

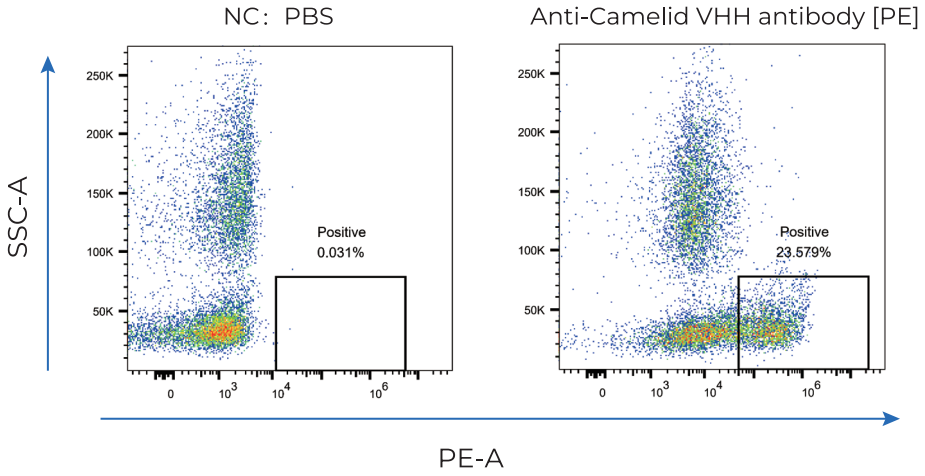
FACS validation

Detection of VHH-based CAR-T cells



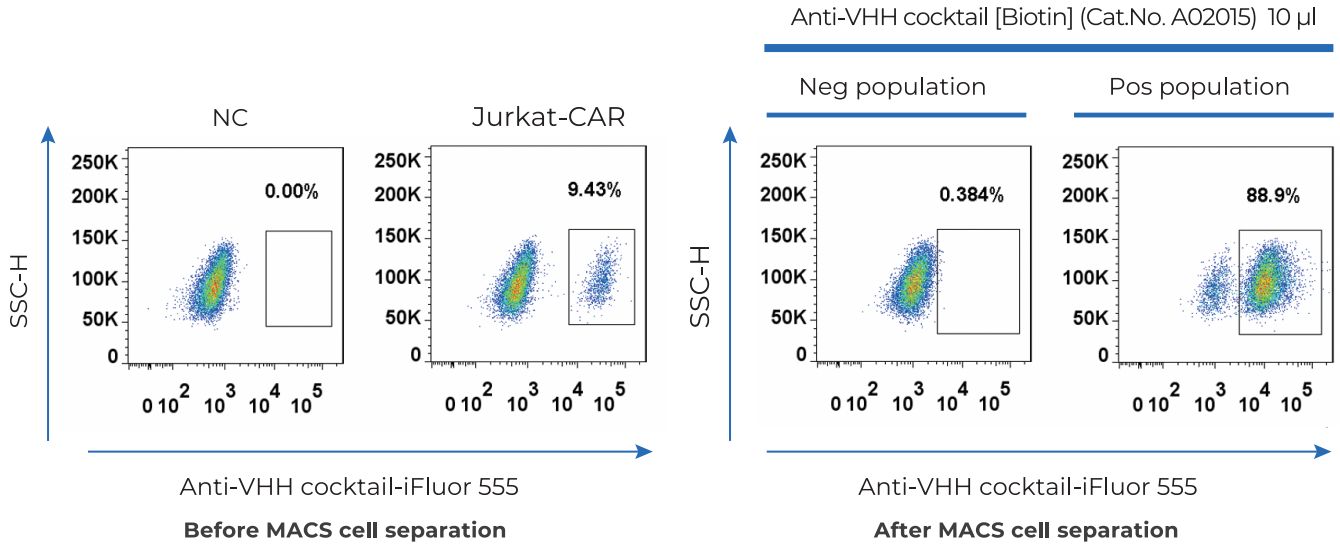
The above data shows FACS binding of a series of dilutions of MonoRab™ Rabbit Anti-Camelid VHH Cocktail [PE] (Cat. No. A02018) with Jurkat cells and samples containing 20% of VHH-based Jurkat-CAR cells. With the development of CAR-T therapy, VHH is often used in the CAR generation. A02018 shows excellent binding activity on VHH-based Jurkat-CAR cells. It is used in CAR-T cell verification and determination.

Selection of VHH-expressing PBMC



VHH-positive PBMCs of non-immunized camel were sorted by FACS using 2 μg MonoRab™ Rabbit Anti-Camelid VHH Cocktail [PE] (Cat.No. A02018). As shown in the figure, the proportion of VHH-positive PBMC cells was 23.579%, which was consistent with the theoretical value, indicating that A02018 has good specificity for VHH-positive PBMCs. A02018 is ideal to be used for isolation of VHH-positive PBMCs.

MACS validation

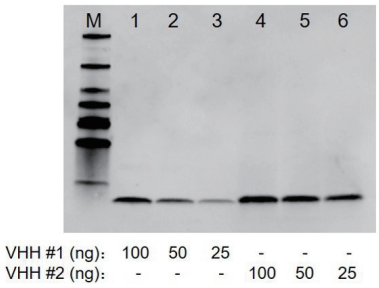


VHH-based CAR-T cells were sorted by MACS with MonoRab™ Rabbit Anti-Camelid VHH Cocktail [Biotin] (Cat. No. A02015) and anti-Biotin magnetic nanobeads (Miltenyi) where naïve T cells serve as negative control. Then, the CAR-T cells were stained with MonoRab™ Rabbit Anti-Camelid VHH Cocktail [iFluor 555] (Cat. No. A02020) and analyzed by FACS.

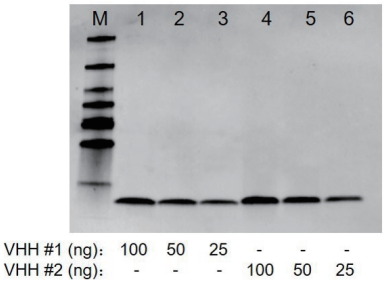
As shown in the figure, the ratio of positive CAR-T cells grows from about 10% to 90% after MACS cell separation. A02015 is ideal to be used for CAR-T cell MACS separation.

WB validation

Western Blot of A01860 with VHHs



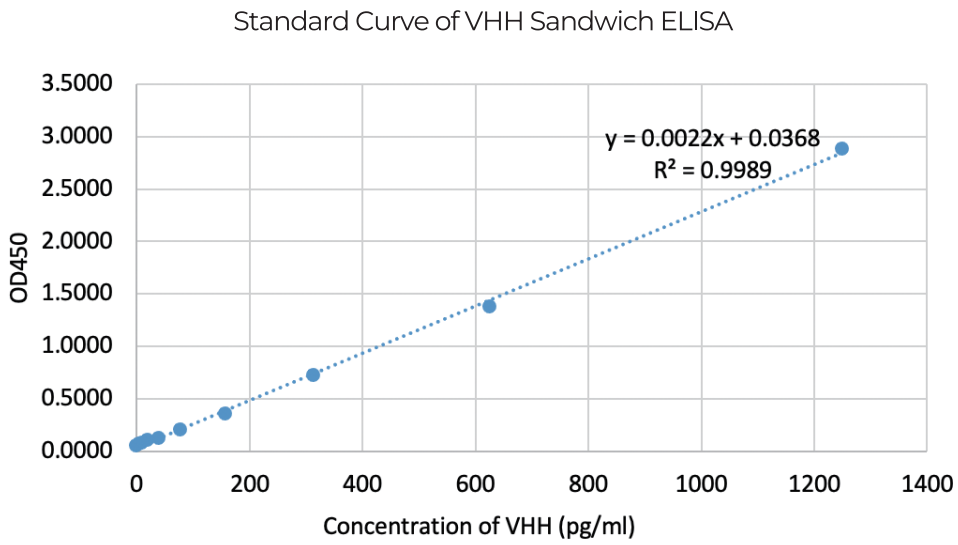
Western Blot of A02014 with VHHs



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 of camelid VHHs with MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014). The results showed that both antibodies had high sensitivity to VHHs.

Sandwich ELISA validation



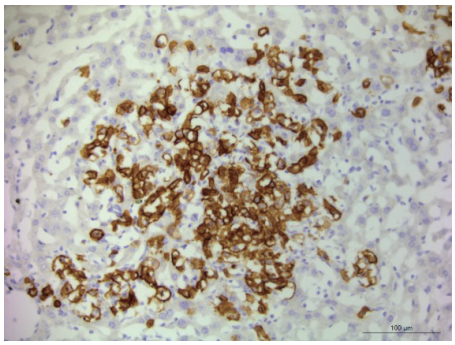
• Standard curve of VHH Sandwich ELISA. The VHH Sandwich ELISA assay is developed using MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (Cat. No. A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail [HRP] (Cat. No. A02016) as the capture and detection antibodies, respectively.

• In this ELISA assay, the detection antibody was labeled with horseradish peroxidase (HRP)*. The sensitivity of detecting VHH is up to 39 pg/ml.

* If your assay needs an unconjugated antibody, GenScript's MonoRab™ Rabbit Anti-Camelid VHH Cocktail is the ideal choice (Cat. No. A02014). GenScript provides customized conjugation services for this product per the customer's request.

IHC validation

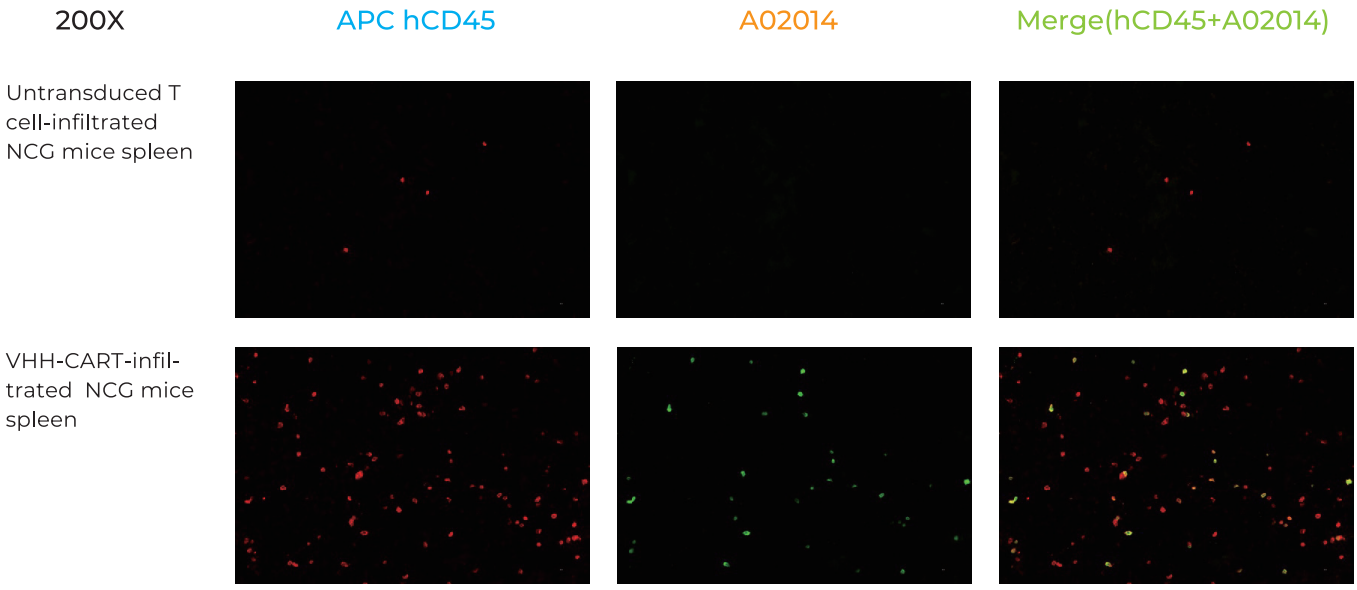
VHH-CART infiltrated liver



• Immunohistochemical analysis of VHH-transduced CAR-T cells infiltrated liver tissue of PDX mouse. VHH was labelled with MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) at 1 μg/ml, followed by incubation of biotinylated anti-rabbit IgG (H+L) (2.5 μg/ml). The final signal was amplified by ABC (Avidin-Biotin Complex) Kit.

Positive staining was shown in the membrane or cytoplasm of VHH-transduced CAR-T cells infiltrated into liver tissue. Counter-stained with hematoxylin.

IF validation



- Immunofluorescence analysis of VHH-transduced CAR-T cells infiltrated NCG mouse spleen, VHH was labelled with MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat. No. A02014) at 0.5 μg/ml, and anti-rabbit IgG (H+L) (Alexa Fluor 488) was used as secondary antibody at 1/500 (4 μg/ml) (Green).
- APC hCD45 monoclonal antibody was used to define all infused T cells at 1/50 (0.5 μg/ml) (Red).
- Confocal images showed cytoplasmic staining in infiltrated VHH-transduced CAR-T cells in the NCG mouse spleen. The untransduced infiltrated T cells showed a negative signal with A02014.

Related Products

L00905 MonoRab™ Anti-Camelid VHH Affinity Resin

GenScript MonoRab™ Anti-Camelid VHH Affinity Resin (Cat. No. L00905) is used to purify camelid VHH from commonly used protein expression systems such as bacteria, yeast and mammalian cells. The VHH in the cell lysates can specifically bind to the anti-camelid VHH monoclonal antibody coupled to the resin, and elute the target protein with high recovery after removal of impurities by stringent washing steps.

Why should you choose Anti-VHH Resin?

In the early discovery phase of VHH development, VHH can be linked to a tag or human Fc, making it easy to express, purify and screen, for example, using a protein A resin for purification. However, at the drug development stage, VHH is essentially not allowed to include tags for safety reasons.

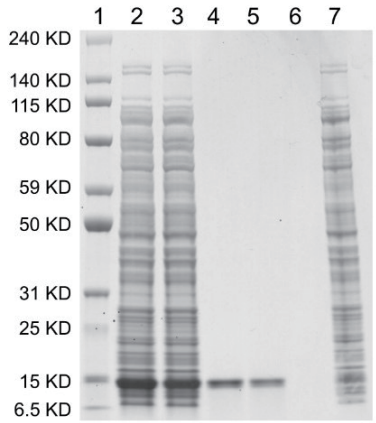
Protein A has high affinity for conventional IgG, but in the development of camelid VHH drugs, if protein A is used as a ligand to purify label-free VHHs, it can only bind to partially wild-type or humanized camelid VHH. In contrast to protein A resin, GenScript's Anti-VHH resin uses MonoRab™ rabbit anti-camelid VHH antibody as an affinity ligand that can target almost all species of wild-type camelid VHH and some humanized camelid VHH, and is not dependent on Fc-tag or His-tag. GenScript's Anti-VHH resin has good binding capacity and specificity, making it a good choice for preclinical studies of VHH drugs.

Characteristics of Anti-Camelid VHH Affinity Resin

Product content	50% settled resin in TBS with 0.02% sodium azide
Matrix	4% cross-linked agarose
Average bead size	90 μm
Ligand	MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb
Binding capacity	Approximately 2-4 mg Camelid VHH protein per mL settled resin (depending on amino acid sequence of different Camelid VHHs)
Storage and stability	Store at 2-8 °C for up to 12 months. Do not freeze the resin.
Resin reuse	When stored at 2 - 8° C, the resin can be recycled at least 5 times with no loss of binding capability. The resin can be reused up to 10 times with minimal loss of binding capability.
Elution method	Acid buffer elution (pH 2.5)
Reagents compatibility	Compatible with commonly used bacterial lysis reagents. For reagents compatible with purification, see tables 2 and 3 in the manual.

Anti-VHH Resin Application Examples

Application example 1: Purification of Camelid VHH from *E. coli* lysate



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

Lane 2: Flow through 1

Lane 3: Flow through 2

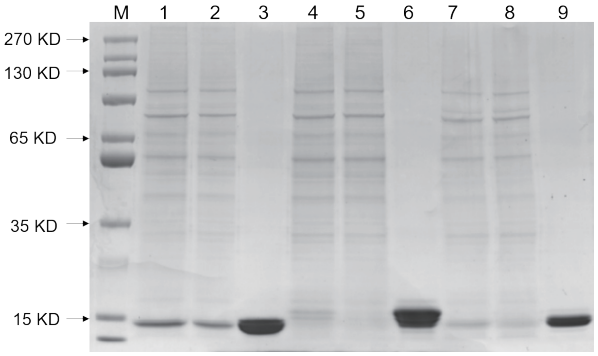
Lane 4: Eluate 1 of target Camelid VHH (M.W.=15 kDa)

Lane 5: Eluate 2 of target Camelid VHH (M.W.=15 kDa)

Lane 6: Empty elution buffer

Lane 7: Whole cell lysate

Application example 2: Purification of humanized VHH from cell culture



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

Lane 1: 1# Humanized VHH cell culture

Lane 4: 2# Humanized VHH cell culture

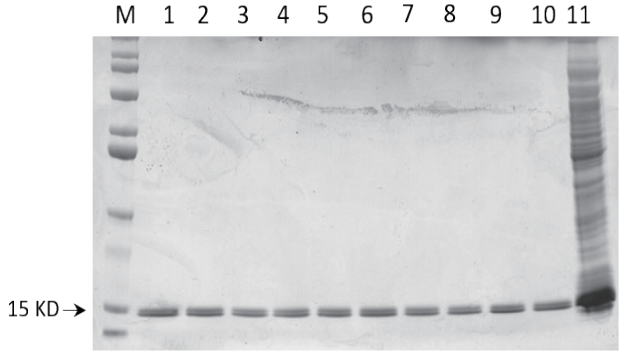
Lane 7: 3# Humanized VHH cell culture

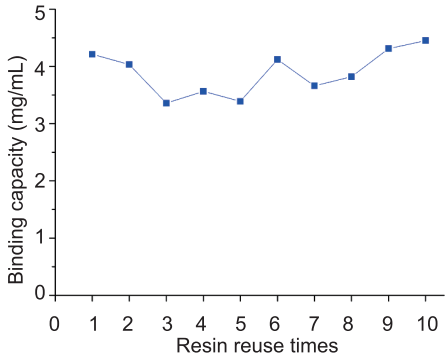
Lane 2, 5, 8: Flow through

Lane 3, 6, 9: Eluted humanized VHH

CONCLUSION: MonoRab™ Anti-Camelid VHH Affinity Resin can be used to purify not only camelid VHH antibodies, but also some humanized VHH antibodies (with limited selectivity for humanized VHH antibody sequence).

Application example 3: The repeat use ability





CONCLUSION: The resin can be reused 10 times with minimal loss of binding capacity.

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