

MonoRab™ Rabbit Anti-Camelid VHH Cocktail

Cat. No.: A02014

Overview

Specificity	GenScript's MonoRab™ Rabbit Anti-Camelid VHH Cocktail is specific for Camelid VHH antibody (Llama, Alpaca, and Camel). It has no cross-reactivity with mouse, rat, rabbit, goat or human immunoglobulins.
Host Species	Rabbit
Immunogen	Llama VHH antibody
Conjugate	Unconjugated

Applications

Working concentrations for specific applications should be determined by the investigators. The appropriate concentrations may be affected by primary/secondary antibody affinity, antigen concentration, the sensitivity of the method of detection, temperature, the length of the incubations, and other factors. The suitability of this antibody for applications other than those listed below has not been determined. The following concentration ranges are recommended starting points for this product. Optimal working dilutions must be determined by the end user.

Application	Recommended Usage
ELISA	1:5,000-1:10,000
Western Blot	1:1,000-1:5,000
Sandwich ELISA	A01860 (C)-A02014 (D)
Immunohistochemistry (IHC)	1 µg/ml
Immunocytochemistry/Immunofluorescence (ICC/IF)	0.5 µg/ml

Properties

Form	Liquid
Storage Buffer	Supplied in PBS (pH 7.4) with 0.02% sodium azide and 50% glycerol.
Concentration	0.5 mg/mL

Storage Instructions	Store at -20°C. This product is stable for 1 year upon receipt, when handled and stored as instructed.
Purification	Protein A affinity column
Isotype	Rabbit IgG
Clonality	Cocktail
Note	GenScript can customize this product per customer's request including product size, buffer components, etc.

Examples

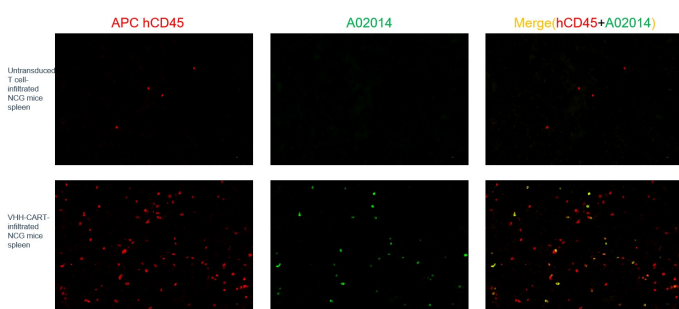


Figure 1. The specificity of MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) to VHH-transduced CAR-T cells was tested by immunocytochemical/immunofluorescence analysis of VHH-transduced CAR-T cells infiltrated NCG mouse spleen.

VHH was labelled with MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) at 0.5 µg/mL, and anti-rabbit IgG (H+L) (Alexa Fluor 488) was used as secondary antibody at 1/500 concentration (4 µg/mL; green). APC hCD45 monoclonal antibody was used to define all infused T cells at 1/50 concentration (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 MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014).

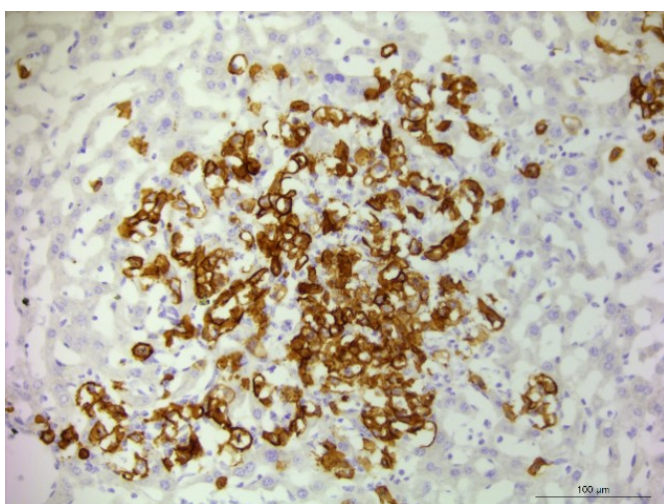
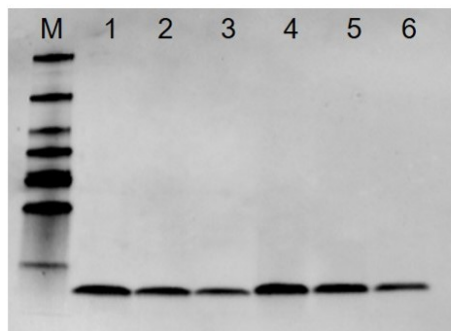


Figure 2. The specificity of MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) to VHH-transduced CAR-T cells was tested by Immunohistochemical analysis of VHH-transduced CAR-T cells infiltrated liver tissue of PDX mouse. VHH was labelled with MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) at 1 µg/mL, followed by incubation with 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. Counterstained with hematoxylin.

Western Blot of A02014 with VHHs



VHH #1 (ng):	100	50	25	-	-	-
VHH #2 (ng):	-	-	-	100	50	25

Figure 3. The affinity of MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014) for different VHHs was analyzed by Western blotting using MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014, 0.5 µg/mL). The signal was developed with Goat Anti-Rabbit IgG Antibody (H&L) [HRP], pAb (Genscript, A00098, 1:5,000).

Lanes 1-3: 100 ng, 50 ng, 25 ng VHH #1

Lanes 4-6: 100 ng, 50 ng, 25 ng VHH #2

ELISA binding of A02014 with different subclasses camelid IgG

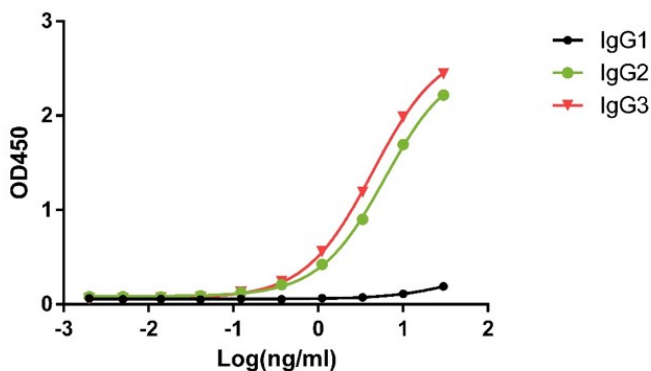


Figure 4. The specificity of MonoRab™ Rabbit Anti-Camelid VHH Cocktail (A02014) to camelid IgG1, IgG2, and IgG3 was tested using ELISA. The results show that MonoRab™ Rabbit Anti-Camelid VHH Cocktail (A02014) is specific to camelid heavy chain antibodies (IgG2 & 3), but not to the camelid conventional antibody (IgG1).

Cross reactivity of A02014 with IgG of different species

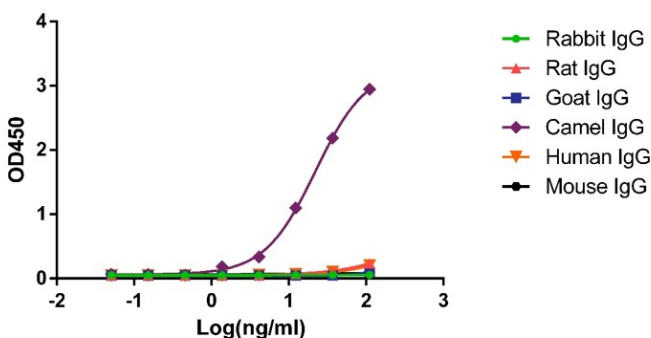


Figure 5. The specificity of MonoRab™ Rabbit Anti-Camelid VHH Cocktail (A02014) to non-camelid IgG was tested using ELISA. The results show that MonoRab™ Rabbit Anti-Camelid VHH Cocktail (A02014) is specific to camelid IgG and has no cross-reactivity with mouse, rat, rabbit, goat and human immunoglobulins.

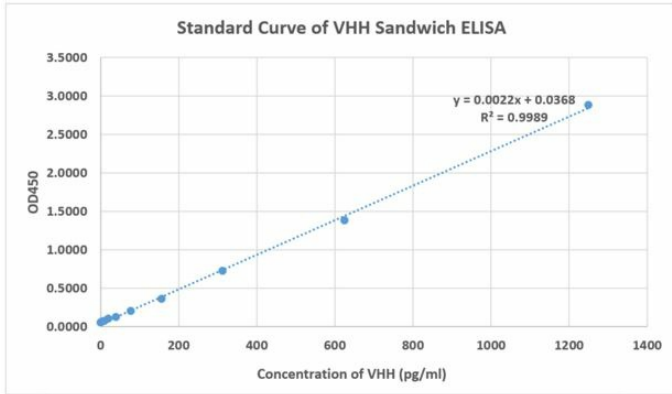


Figure 6. Standard curve for quantitative detection of VHH by sandwich ELISA. The VHH Sandwich ELISA assay is developed by using MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb (GenScript, A01860) and MonoRab™ Rabbit Anti-Camelid VHH Cocktail [HRP] (GenScript, A02016) as the capture and detection antibodies, respectively. In this ELISA assay, the detection antibody was labeled with horseradish peroxidase (HRP). However, of an unconjugated detection antibody is desired, MonoRab™ Rabbit Anti-Camelid VHH Cocktail (A02014) can be used for this assay.

Note: GenScript can provide customized conjugation services for this product as per customer's request.

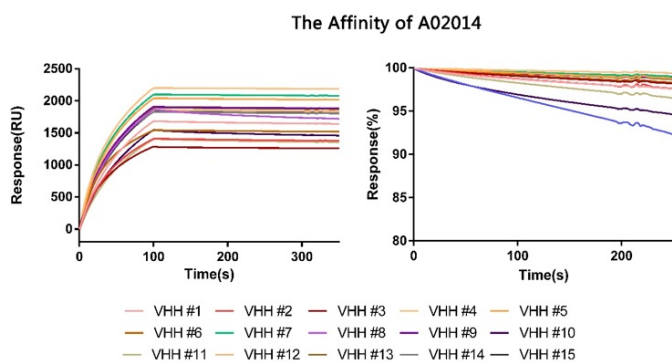


Figure 7. The affinity of MonoRab™ Rabbit Anti-Camelid VHH Cocktail (Cat.no A02014) with 15 random VHHs was measured by Biacore. The cocktail antibody shows comprehensive binding activity and high affinity with all of the 15 VHHs.

Background

Target Background : A camelid single-domain antibody (sdAb, called Nanobody by Ablynx, the developer) is a peptide chain about 110 amino acids long, comprising one variable domain of a heavy-chain antibody, these are called VHH fragments. Like a whole antibody, it is able to bind selectively to a specific antigen. Single-domain antibodies allow a broad range of applications in biotechnical as well as therapeutic fields due to their small size, simple production and high affinity.

Synonyms : Rabbit anti-camelid VHH, single-domain antibody monoclonal antibody cocktail (Min X Ms, Rt, Rb, Gt, Hu).

For laboratory research use only. Direct human use, including taking orally and injection and clinical use are forbidden.