

MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate

Version: 01
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Product Name	Cat.No	Size
MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate (Clear, 8×12 strip)	L00946	5 plates

The product is used for rapid capture of VHHs in different samples.

The operator should read technical manual carefully before using this product.
For research use only. Not for use in diagnostic procedures

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I. Description

Camelid VHH Antibody (also called single-domain antibody (sdAb), or Nanobody by Ablynx) is a peptide chain about 110 amino acids in length, containing one variable domain of a heavy-chain antibody (a VHH fragment). Like a whole antibody, the camelid VHH antibody is able to bind selectively to a specific antigen. Due to its small size, simple production and high affinity, the camelid VHH antibody has a broad range of applications in biotechnical and therapeutic research fields.

MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate (Clear, 8×12 strip) (GenScript, L00946) is a 96-well microtiter plate coated with MonoRab™ Rabbit Anti-Camelid VHH Cocktail (GenScript, A02014). It can bind camelid and humanized VHHs with high specificity and capacity. The binding capacity and detection limits of plate varies, depending on the amino acid constitution of different VHH. The plate can be compatible with MonoRab™ Rabbit Anti-Camelid VHH Cocktail [HRP] (GenScript, A02016), HRP (Horseradish peroxidase) conjugated secondary antibody, or HRP-conjugated specific antigen. Any detection reagent, which can recognize Rabbit IgG, **should not be** applied to the plate, such as HRP-conjugated Mouse anti-Rabbit IgG and HRP-Protein A.

There are several potential applications for the plate:

- Fix anti-VHH antibodies on the plate, which provides a universal platform to monitor the VHH level in different culture condition and to detect specific VHH for immunogenicity studies.
- High throughput screening of stable cell lines expressing camelid or humanized VHHs.
- Study the pharmacokinetics of camelid or humanized VHH antibody drugs in serum.
- Detect the immuno-response of the heavy-chain-only antibody in camelid after immunization.

II. Key Features

Features	Specifications
Pre-coated Antibody	MonoRab™ Rabbit Anti-Camelid VHH Cocktail
Specificity	Camelid (Alpaca and Llama) and humanized VHHs
Sensitivity	1 ng/ml
Binding capacity	~3 ng/well
Reagents compatibility	Compatible with common used reagents (see Reagent Compatibility table)

III. Storage

MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate should be shipped on ice pack. The unopened plate is stable for at least 1 years when stored at 2-8 °C. The opened plate should be used within one week.

IV. Instructions for use

- All the reagents should be equilibrated to room temperature (20-25 °C) before test.
- This manual gives general protocols for different assays. The user should optimize the protocol to achieve ideal test result.
- The user should count the strips of MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate required for an assay and leave the unused strips in the foil pouch and store at 2-8 °C.

1. VHHs Expression Screening

- This procedure utilizes sandwich ELISA method to perform protein expression screening in samples.
- Other enzyme substrate developing system besides HRP-TMB could also be used in this application.

Materials and Equipment:

- **Provided in the Kit**

MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate

Plate sealer

- **Not provided in the Kit**

Samples containing VHHs

HRP-conjugated secondary antibody or antigen against target protein

TMB Substrate; Stop Solution

1 × Washing Solution (1 × PBST)

Pipette

Microplate reader capable of measuring absorbance at 450 nm.

Procedure Guideline:

- Add 100 µL of test samples, negative control or positive control to different wells of MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate;

- ii. Cover the plate with plate sealer and incubate at 37°C for 30 min;
- iii. Wash the plate with 260 µL/well of 1 × Washing Solution for four times;
- iv. Add 100 µL of prepared HRP-conjugated secondary antibody against target protein to each well;
- v. Cover the plate with plate sealer and incubate at 37°C for 30 min;
- vi. Wash the plate with 260 µL/well of 1 × Washing Solution for four times;
- vii. Add 100 µL of TMB Substrate to each well and incubate at room temperature for 15 min;
- viii. Add 50 µL of Stop Solution to each well to stop the reaction;
- ix. Read absorbance of the plate on a microplate reader at 450 nm

2. VHHs Quantification

- This procedure utilizes sandwich ELISA method to quantify protein expression in samples.
- Before test, the researcher should do preliminary experiment to set up a standard curve for their VHHs.
 - Other enzyme-substrate developing system besides HRP-TMB could also be used in this application.

Materials and Equipment:

- **Provided in the Kit**

MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate

Plate sealer

- **Not provided in the Kit**

Samples containing VHHs

VHH standard

HRP-conjugated antibody against VHH (Detection antibody)

TMB Substrate; Stop Solution

1 × Washing Solution (1 × PBST)

Pipette

Microplate reader capable of measuring absorbance at 450 nm.

Procedure Guideline:

- i. Add 100 µL of test samples and prepared VHH standard to different wells of MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate (duplicate for each sample and standard is recommended);
- ii. Cover the plate with plate sealer and incubate at 37°C for 30 min;
- iii. Wash the plate with 260 µL/well of 1 × Washing Solution for four times;
- iv. Add 100 µL of prepared HRP-conjugated antibody against VHH to each well;
- v. Cover the plate with plate sealer and incubate at 37°C for 30 min;
- vi. Wash the plate with 260 µL/well of 1 × Washing Solution for four times;

- vii. Add 100 μ L of TMB Substrate to each well and incubate at room temperature for 15 min;
- viii. Add 50 μ L of Stop Solution to each well to stop the reaction;
- ix. Read absorbance of the plate on a microplate reader at 450 nm
- x. Generate a standard curve by plotting the average absorbance on the Y axis versus the corresponding **log concentration** of Camelid VHH antibody standard on the X axis, fitting with the four-parameter equation.
- xi. The amount of VHHs in each sample is determined by extrapolating OD values to the standard curve.

Note: The procedures above are designed by using **MonoRab™ Rabbit Anti-Camelid VHH Cocktail [HRP] (GenScript, A02016)** as the detection antibody, and the detection range of MonoRab™ Rabbit Anti-Camelid VHH Antibody Plate in this condition is around 1-10 ng/ml. As for other detection method, the protocol may need be optimized.

V. Reagents compatibility table

Some reagents may interfere with the test results. Check the reagents concentration in samples according to the Reagent compatibility table. Dialysis or dilute samples if needed.

Reagent	Compatible concentration
Triton X-100	\leq 5%
NP-40	\leq 4%
Guanidine HCl	\leq 200 mM
Urea	\leq 0.5 M
SDS	\leq 0.3%
DTT	\leq 1 mM
Tween-20	\leq 5%
NaCl	\leq 1 M
TBS	Compatible
PBS	Compatible

VI. Troubleshooting

Problem	Probable Cause	Solution
No signal	HRP-conjugated secondary antibody against target protein do not pair with coated anti-Camelid VHH cocactail antibody	Choose other HRP-conjugated secondary antibody or HRP-conjugated antigen as alternative
High signal	The concentration of VHH is too high in sample	Dilute the sample
Weak signal	HRP-conjugated secondary antibody against target protein do not pair with coated anti-Camelid VHH cocactail antibody	Choose other HRP-conjugated secondary antibody or HRP-conjugated antigen as alternative
	The concentration of VHH is too low in sample	Concentrate sample
	The working concentration of HRP- conjugated secondary antibody is low	Increase the working concentration of HRP-conjugated secondary antibody
	The secondary antibody incubation time is short	Increase secondary antibody incubation time
	Substrate incubation time is short	Increase substrate incubation time
	Incompatible reagent(s) concentration	Check and decrease concentration of reagent(s) in sample
High Background	The working concentration of HRP- conjugated secondary antibody is high	Decrease the working concentration of HRP-conjugated secondary antibody
	Insufficient washing	Increase washing times
	Washing Solution is polluted	Use new prepared Washing Solution
	Substrate incubation time is too long	Decrease substrate incubation time

VII. Related Products

Cat. No.	Product Name
A01860	MonoRab™ Rabbit Anti-Camelid VHH Antibody, mAb
A01995	MonoRab™ Rabbit Anti-Camelid VHH Antibody [Biotin], mAb
A01861	MonoRab™ Rabbit Anti-Camelid VHH Antibody [HRP], mAb
A02165	MonoRab™ Rabbit Anti-Humanized VHH Antibody, mAb
A02166	MonoRab™ Rabbit Anti-Humanized VHH Antibody [Biotin], mAb
A02167	MonoRab™ Rabbit Anti-Humanized VHH Antibody [HRP], mAb
A02014	MonoRab™ Rabbit Anti-Camelid VHH Cocktail
A02015	MonoRab™ Rabbit Anti-Camelid VHH Cocktail [Biotin]
A02016	MonoRab™ Rabbit Anti-Camelid VHH Cocktail [HRP]

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GenScript USA Inc.

860 Centennial Ave.,

Piscataway, NJ 08854

Tel: 1-877-436-7274, 1-732-885-9188

Fax: 732-210-0262, 732-885-5878

Email: product@genscript.com

Web: www.genscript.com

生产商: 南京金斯瑞生物科技有限公司 江苏省南京市江宁区科学园雍熙路28号

Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China