MonoRab™ Rabbit Anti-scFv Cocktail

Cat. No.: A02282

Overview

Specificity	This product is specific for scFvs in different species (humanized, mouse) and different orders (VH-linker-VL, VL-linker-VH). It can also recognize various forms of scFv-based bispecific antibodies, such as DART, BiTE, TandAbs, IgG(H)-scFv2, scFv-Fab-Fc, etc.
Host Species	Rabbit
Immunogen	This information is confidential and belongs to GenScript.
Conjugate	Unconjugated

Applications

Working concentrations for specific applications should be determined by the investigators. The appropriate concentrations may be affected by 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.

Application	Recommended Usage
ELISA	0.05-0.1 µg/ml
Flow Cytometry	1 µg/ml-10 µg/ml
Western Blot	0.05-0.5 µg/ml

Properties

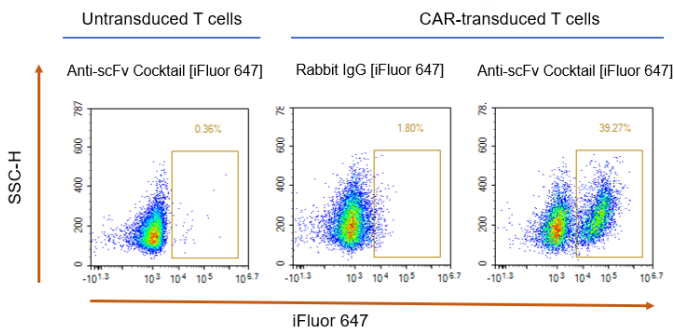|	
Form	Liquid
Storage Buffer	Supplied in PBS (pH 7.2), 0.02% sodium azide and 50% glycerol.
Concentration	0.5 mg/ml
Storage Instructions	Store at -20°C. This product is stable for 1 year upon receipt, when handled and stored as instructed.
Purification	Protein A affinity column

Isotype	Rabbit IgG, κ
Clonality	Monoclonal antibody cocktail
Note	GenScript can customize this product per customer's request including product size, buffer components, etc.

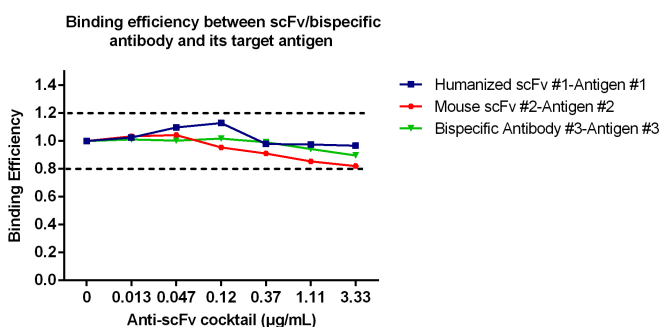# Examples

Anti-Human IgG F(ab')2 · Protein L · A02282
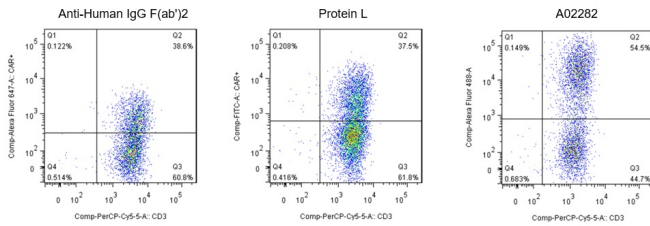
Flow cytometric analysis of scFv-based CAR-T cells using different characterization methods.

Live pan-CD3+ T cells were isolated from human PBMCs and engineered to express an scFv-based CAR. MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282, 10μg/mL) demonstrated the highest positivity rate and the best cell clustering compared to concentration-matched FITC-Labeled Recombinant Protein L and Alexa Fluor® 647 AffiniPure Goat Anti-Human IgG, F(ab ')2.The signal was amplified using Alexa Fluor® 488 -Labeled Goat Anti-Rabbit IgG.

Untransduced T cells — CAR-transduced T cells
Anti-scFv Cocktail [iFluor 647] · Rabbit IgG [iFluor 647] · Anti-scFv Cocktail [iFluor 647]

Flow cytometric analysis of scFv-based CAR-T cells using MonoRab™ Rabbit Anti-scFv Cocktail [iFluor 647] (GenScript, A02288). The untransduced T cells and concentration-matched Rabbit IgG [iFluor 647] (GenScript, A02026) were used as controls.
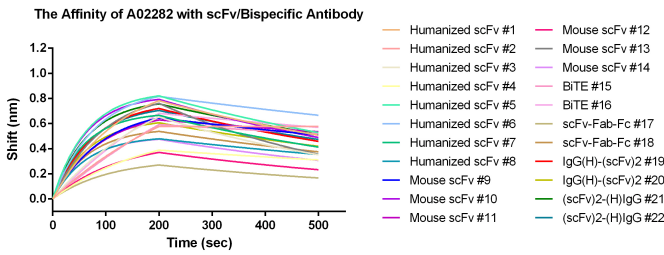
The results show that MonoRab™ Rabbit Anti-scFv Cocktail can achieve good cell clustering for CAR-transduced T cells and show minimal non-specificity for untransduced T cells.

Binding efficiency between scFv/bispecific antibody and its target antigen

- Humanized scFv #1-Antigen #1
- Mouse scFv #2-Antigen #2
- Bispecific Antibody #3-Antigen #3

MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) exhibited non-inhibitory properties for the binding between scFv/bispecific antibody and its target antigen.

In this ELISA assay, the target antigen was coated at a concentration of 1 μg/mL, and scFv was mixed with A02282 (with dilutions starting from 3.33 μg/mL) before being added to the antigen-coated plates. Subsequently, THE™ His Tag Antibody [HRP], mAb, Mouse (GenScript, A00612) was utilized as the detection antibody at a dilution of 1:5000.

Humanized scFv#1: anti-VEGF scFv; Antigen#1: VEGF; Mouse scFv#2: anti-BCMA scFv; Antigen#2: BCMA; Bispecific Antibody#3: scFv based anti-CD19×anti-CD3 bispecific antibody; Antigen#3: CD3.

The Affinity of A02282 with scFv/Bispecific Antibody

Humanized scFv #1	Mouse scFv #12
Humanized scFv #2	Mouse scFv #13
Humanized scFv #3	Mouse scFv #14
Humanized scFv #4	BiTE #15
Humanized scFv #5	BiTE #16
Humanized scFv #6	scFv-Fab-Fc #17
Humanized scFv #7	scFv-Fab-Fc #18
Humanized scFv #8	IgG(H)-(scFv)2 #19
Mouse scFv #9	IgG(H)-(scFv)2 #20
Mouse scFv #10	(scFv)2-(H)IgG #21
Mouse scFv #11	(scFv)2-(H)IgG #22

The affinity of MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) with different sequences of scFv was measured by BLI (Biolayer Interferometry).

MonoRab™ Rabbit Anti-scFv Cocktail shows high affinity with scFv in different species (humanized, mouse), different linker (GS linker or whitlow/218 linker) and different orders (VH-linker-VL, VL-linker-VH). It also shows high affinity with various forms of scFv-based bispecific antibodies, such as BiTE, IgG(H)-(scFv)2, (scFv)2-(H)IgG, scFv-Fab-Fc, etc.

Humanized scFv#1: VL-(G4S)3 linker-VH; Humanized scFv#2: VH-Whitlow/218 linker-VL; Mouse scFv#9: VL-Whitlow/218 linker-VH; Mouse scFv#10: VH-(G4S)3 linker-VL.
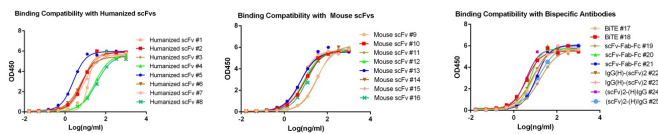

The binding compatibility of MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) with different sequences of scFv or scFv-based bispecific antibodies was detected by ELISA.

The results show that MonoRab™ Rabbit Anti-scFv Cocktail is specific for scFvs in different species (humanized, mouse), different linker (GS linker or whitlow/218 linker) and different orders (VH-linker-VL, VL-linker-VH). It can also recognize various forms of scFv-based bispecific antibodies, such as BiTE, IgG(H)-(scFv)2, (scFv)2-(H)IgG, scFv-Fab-Fc, etc.

Humanized scFv#1: VL-(G4S)3 linker-VH; Humanized scFv#2: VH-Whitlow/218 linker-VL; Mouse scFv#9: VL-Whitlow/218 linker-VH; Mouse scFv#10: VH-(G4S)3 linker-VL.

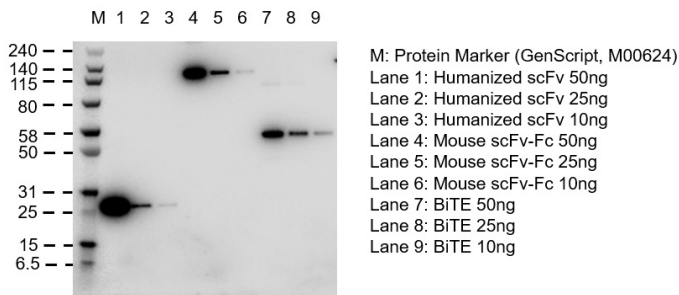## Binding curve between scFv/bispecific antibody and its target antigen detected by A02282
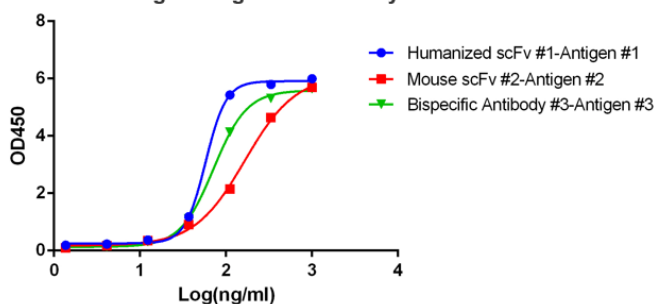
The binding curve between scFv/bispecific antibody and its target antigen was detected using MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282) in an ELISA assay.

In this ELISA assay, the target antigen was coated at a concentration of 1 µg/mL, and scFv was utilized within a concentration range from 1.37 ng/mL to 1000 ng/mL. Subsequently, A02282 was used as a detection antibody at a concentration of 1 µg/mL, and the signal was amplified using Mouse Anti-Rabbit IgG Fc Antibody (14H9H10) [HRP], mAb (GenScript, A01856) at a dilution of 1:20,000.

Humanized scFv#1: anti-VEGF scFv; Antigen#1: VEGF; Mouse scFv#2: anti-BCMA scFv; Antigen#2: BCMA; Bispecific Antibody#3: scFv based anti-CD19×anti-CD3 bispecific

antibody; Antigen#3: CD3.

240 —
140 —
115 —
80 —
58 —
50 —
31 —
25 —
15 —
6.5 —

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 analysis of humanized scFv, mouse scFv-Fc and bispecific antibody (BiTE) at various concentrations was performed using MonoRab™ Rabbit Anti-scFv Cocktail (GenScript, A02282, 0.1 µg/mL). The signal was detected using Mouse Anti-Rabbit IgG Fc Antibody (14H9H10)[HRP], mAb, (GenScript, A01856, 1:20,000).

Background

Target Background : 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. 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 cell therapy, scFvs can act as the antigen recognition domain of CAR-T cells and determine the targeting ability of these cells.

Synonyms : scFv; Single-chain fragment variable; Nanobody; Single chain antibody; VH and VL of an antibody; Single-chain variable region fragment; Single-chain fv; VH-linker-VL; VL-linker-VH

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