Anti-Human IgG Fab, AlpSdAbs® VHH (VcMMAE ×4)

Summary

Code	023-102-101
Immunogen	Recombinant human IgG
Host	Alpaca pacous
Isotype	VHH domain of alpaca IgG2b/2c
Conjugate	VcMMAE(2 moles VcMMAE per mole VHH)
Specificity	Human IgG Fab
Cross-Reactivity	Recognizes human IgG Fab specifically, and reacts with cynomolgus IgG. No Cross-reactivity to rabbit , mouse, rat, goat IgG
Purity	Recombinant Expression and Affinity purified
Concentration	0.5mg/ml
Formation	Liquid, 10mM PBS (pH 7.4)
Storage	Store at –20 °C(Avoid freeze / thaw cycles)

Description

Anti-Human IgG Fab, AlpSdAbs® VHH(VcMMAE ×4) is designed for studying on the internalization of antibodies. Anti-Human IgG Fab, AlpSdAbs® VHH(VcMMAE ×4) is based on monoclonal, recombinant single domain antibody to human IgG Fab coupled to VcMMAE. Based on immunoelectrophoresis and/or ELISA, Anti-Human IgG Fab, AlpSdAbs® VHH(VcMMAE ×4) reacts with the human IgG Fab. Anti-Human IgG Fab, AlpSdAbs® VHH(VcMMAE ×4) is an effective detection tool and can be used as a useful tool for the evaluation of antibody potency prior to ADCs.

Background

VHH are single-domain antibodies derived from the variable regions of heavy chain of Camelidae immunoglobulin. The size of VHH is extremely small(<15KDa) compared to other forms of antibody fragment, which significantly increase the permeability of VHH. Thus VHH is considered of great value for research, diagnostics and therapeutics.

Benefits

High lot-to-lot consistency
Increased sensitivity and higher affinity
Animal-free production

Application notes

Antibody Internalization Test: 2ug per 10ug test antibody(molar ratio=2:1).

Dilution factors are presented in the form of a range because the optimal dilution is a function of many factors, such as antigen density, permeability, etc. The actual dilution used must be determined empirically.

Protocol

1. Preparation of Test Cells - Day1

1) On Day 1 of the formal experiment, it was confirmed that the cells to be tested are in good condition. After thorough dilution and mixing, the cells are seeded at 1000-5000 cells/well/100uL in a 96 well cell culture plate. The test cells counts need to be adjusted by the user based on the growth rate and characteristics of the selected cells.

2) After inoculation, place the cell culture plate back into the incubator and culture it under suitable conditions.

*Different cells growth rates vary greatly, and it is recommended to lay the board at a density of around 50% for subsequent endocytosis/internalization and killing detection.

2. Preparation of Test Antibody Sample - Day1

1) Preparation of mAb-VHH-MMAE complex solution: Mix the test antibody(mAb) directly with VHH-MMAE in a molar ratio of 1:2 and let them mix thoroughly at room temperature (20-37 °C) for 20 minutes;

*In this step, the test antibody and VHH-MMAE can be mixed directly without dilution, with a concentration range of 0.1mg/mL-10mg/mL.

*The buffer of the test antibody in this step will not affect the binding of VHH-MMAE to form a complex.

2) Preparation of mAb-VHH-MMAE complex solution at working concentration: Based on the user's mAb-VHH-MMAE complex concentration, prepare the test antibody into a 30ug/mL (200nM) solution using cell culture medium and mix evenly.

3) Dilute mAb-VHH-MMAE complex according to experimental requirements: Dilute mAb-VHH-MMAE complex in a gradient using culture medium;

4) Add 100ul of diluted mAb-VHH-MMAE complex to a 96 well cell culture plate, put plate back to the incubator, and culture under suitable conditions for 48-96 hours.

*There are certain differences in the detection and cultivation time of different cells, test antibody and drug.

*Users can design controls group according to experimental needs and actual situations.

3. Killing Test

1) Observe cells growth and remove the culture medium from the cell culture plate before testing.

2) Use ATP content measurement method (such as CTG) to detect cells proliferation and vitality, in order to determine antibody internalization. Please refer to the instruction manual of the corresponding reagent kit for specific operating steps.

The calculation method for antibody internalization rate is:

Internalization rate of test antibody=[(Ac As)/Ac] × 100%;

As: Experimental group absorbance (Test antibody+internalization detection reagent group);

Ac: Control group absorbance (Isotype Control antibody+internalization detection reagent group)