Anti-Mouse IgG, AlpSdAbs® VHH(VcPBD ×4)

Summary

Code	001-101-104
Immunogen	Recombinant mouse IgG
Host	Alpaca pacous
Isotype	VHH domain of alpaca IgG2b/2c
Conjugate	VcPBD(2 moles VcPBD per mole VHH)
Specificity	Mouse IgG(Fcγ fragment specific)
Cross-Reactivity	No cross-reactivity with mouse IgM, rabbit, human, cynomolgus, 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-Mouse IgG, AlpSdAbs® VHH(VcPBD ×4) is designed for studying on the internalization of antibodies. Anti-Mouse IgG, AlpSdAbs® VHH(VcPBD ×4) is based on recombinant single domain antibodies to mouse IgG Fc coupled to VcPBD. Based on immunoelectrophoresis and/or ELISA, Anti-Mouse IgG, AlpSdAbs® VHH(VcPBD ×4) reacts with the Fc fragment of mouse IgG(including mouse IgG1, IgG2a, IgG2b) heavy chain but not with the Fab portion of mouse IgG. Anti-Mouse IgG, AlpSdAbs® VHH(VcPBD ×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.

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-PBD complex solution:
Mix the test antibody(mAb) directly with VHH-PBD in a molar ratio of 1:2 and let them mix thoroughly at room temperature (20-37 ℃) for 20 minutes;
*In this step, the test antibody and VHH-PBD 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-PBD to form a complex.
2) Preparation of mAb-VHH-PBD complex solution at working concentration:
Based on the user's mAb-VHH-PBD complex concentration, prepare the test antibody into a 30ug/mL (200nM) solution using cell culture medium and mix evenly.
3) Dilute mAb-VHH-PBD complex according to experimental requirements:
Dilute mAb-VHH-PBD complex in a gradient using culture medium;
4) Add 100ul of diluted mAb-VHH-PBD 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).