Anti-Mesothelin-Anti-CD3 Bispecific Antibody

Catalog: 101621
Lot: 230308-HA

Product Information

Description:	Anti-mesothelin-Anti-CD3 (Knot-in-Knob) antibody recognizes human mesothelin and CD3 proteins. This human bispecific antibody has been tested for specific binding affinity to mesothelin. The recombinant antibody was affinity purified.
Background:	Bispecific antibodies (BsMab) are chimeric antibodies with the ability to bind to two antigens and are typically created for immune cell activation, interfering with signal pathways or bringing proteins together. Mesothelin is a tumor differentiation antigen present in mesothelioma, ovarian cancer, pancreatic adenocarcinoma and others. CD3 (Cluster of Differentiation 3) is present in T cells and is involved in T cell activation. The ability to bring cancer cells to T cells allows for enhanced tumor cell killing and is a valuable tool in cancer immunotherapy.
Species:	Human
Clonality:	Monoclonal
Concentration:	0.29 mg/ml
Expression System:	HEK293
Purity:	≥90%
Format:	Aqueous buffer solution.
Formulated In:	8 mM phosphate, pH 7.4, 110 mM NaCl, 2.2 mM KCl, and 20% glycerol
MW:	Heavy Chain: 49 kDa; Light Chain: 23 kDa
Stability:	At least 6 months at -80°C.
Storage:	-80°C
Instructions for Use:	Thaw on ice and gently mix prior to use. DO NOT VORTEX. Perform a quick spin before opening. Aliquot into small volumes and flash freeze for long term storage. Avoid multiple freeze/thaw cycles.
Assay Conditions:	The antibody was validated by measuring anti-Mesothelin-anti-CD3 binding to Mesothelin antigen in ELISA. The anti-Mesothelin-anti-CD3 antibody (BPS Bioscience # 101621) was coated onto a 96-well plate overnight at 4°C (50 µl/well at a concentration of 4 µg/ml in PBS). The plate was washed 3 times with Immuno Buffer 1 (BPS Bioscience #79311) and blocked using 100 µl of Blocking Buffer 2 (BPS Bioscience #79728) for 1 hour at room temperature. After removing the blocking buffer, 50 µl/well of purified biotinylated Mesothelin protein (BPS Bioscience #100291), serially diluted in Blocking Buffer 2, was added for 30 minutes at room temperature. The plate was washed, incubated with Streptavidin-HRP, washed again, and incubated with the Colorimetric HRP substrate. The reaction was stopped, and absorbance was read at 450 nm. The Blank value was subtracted from all values.
Applications:	Binding studies to mesothelin and CD3 in biochemical and cellular assays.

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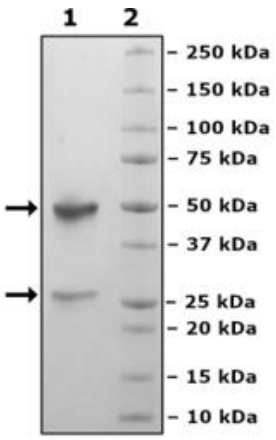
Quality Control Data

4-20% SDS-PAGE Coomassie Staining



Experimental design and assay protocol used for measuring anti-Mesothelin-anti-CD3 functional activity using NFAT-Luc reporter Jurkat cell line:

Jurkat effector cells transfected with the luciferase reporter gene under the control of NFAT (Nuclear Factor of Activator T cells; BPS Bioscience #60621) were incubated with increasing concentrations of anti-Mesothelin x anti-CD3 bispecific antibody in the presence of Mesothelin-CHO cells (BPS Bioscience #78132) or control CHO-K1 cells (ATCC #CCL-61™).

Protocol:

1. CHO and Mesothelin-CHO cells were seeded at 30,000 cells/well and allowed a few hours for the cells to attach in a 96-well clear bottom white plate.

2. NFAT-Luc reporter Jurkat cells were seeded at 30,000 cells/well in co-culture with CHO and Mesothelin-CHO cells.

3. The bispecific antibody was diluted (range of 56.5 fM-10 nM) and added to the cells. The bispecific antibody simultaneously binds to CD3 on the NFAT-Luc Jurkat reporter cells and to Mesothelin on Mesothelin CHO cells. A no-antibody control was included to determine the background signal.

4. After 16 hours, luciferase activity resulting from the activation of NFAT in Jurkat cells was measured using ONE-Step™ luciferase assay (BPS Bioscience #60690) as per the recommended protocol. As shown in the graph below, bispecific antibody engagement to both the Mesothelin-CHO cells and the Jurkat reporter cells stimulated NFAT-luciferase activity.

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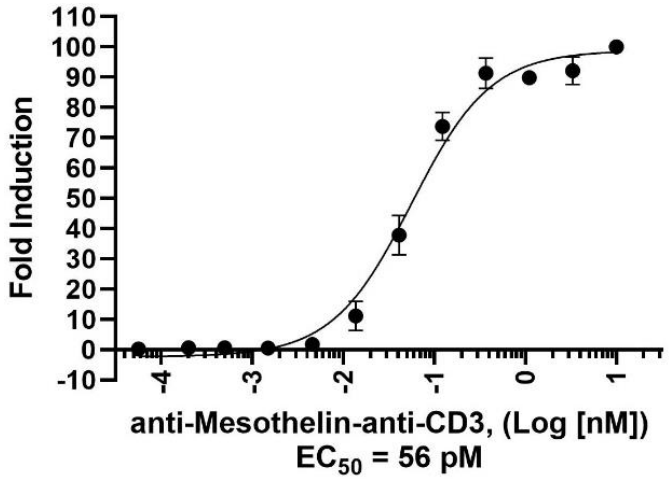
Activation of luciferase in NFAT-Jurkat Reporter cells by Anti-Mesothelin-Anti-CD3 IgG in the presence of Mesothelin-CHO cells



Anti-Mesothelin-Anti-CD3 Binding Assay