Anti-FcGR3A-Anti-EGFR Bispecific Antibody

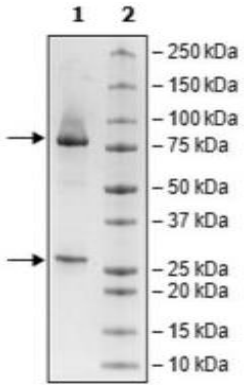
Catalog: 101535
Lot: 220809

Product Information

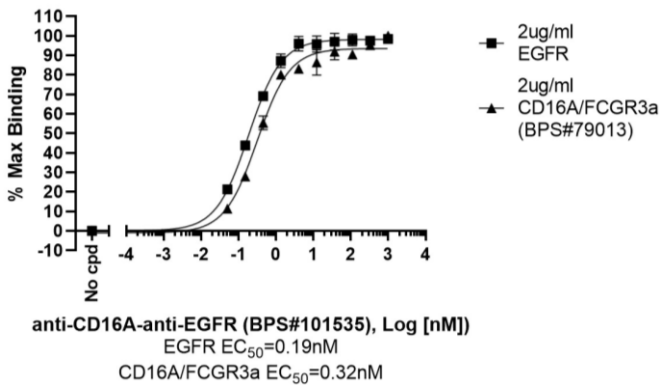
Description:	Anti-FcGR3A-anti-EGFR bispecific IgG antibody, which recognizes FcGR3A (also known as CD16A) and EGFR antigen binding proteins. This bispecific antibody has been tested for specific binding affinity to purified human FcGR3A and EGFR recombinant protein.
Species:	Human
Clonality:	Comprised of two monoclonal antibodies
Concentration:	3.14 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: 76 kDa; Light Chain: 23 kDa + glycans
Glycosylation:	This antibody runs at a higher MW by SDS-PAGE due to glycosylation.
Stability:	At least 12 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:	Functional validation: The antibody was validated by measuring anti-FcGR3A-anti-EGFR binding to FcGR3A and EGFR antigens in ELISA assay. The FcGR3A protein (BPS Bioscience #79013) and EGFR protein were coated onto a 96-well plate overnight at 4°C (50 µl/well at a concentration of 2 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 anti-FcGR3A-anti-EGFR antibody (BPS Bioscience #101535), serially diluted in Blocking Buffer 2, was added for 30 minutes at room temperature. After 3 more washes, the plate was incubated with Goat Anti-Human IgG Fc (HRP) (Abcam #ab97225), washed, 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:	Useful for studying the binding of FcGR3A and EGFR in ELISA and in cellular assays.

Quality Control Data

4-20% SDS-PAGE Coomassie Staining



Binding Assay



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Experimental design and assay protocol used for measuring anti-FcGR3A-anti-EGFR functional activity using FcGR3A/NFAT-luc reporter Jurkat cell line:

Jurkat effector cells expressing endogenous FcGR3A and transfected with the luciferase reporter gene under the control of NFAT (Nuclear Factor of Activator T cells; BPS Bioscience #60541) were incubated with increasing concentrations of anti-FcGR3A x anti-EGFR bispecific antibody in the presence of EGFR-CHO cells (BPS Bioscience #78145-H) or control CHO cells (ATCC #CCL-61™).

Protocol:

1. CHO and EGFR-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. FcGR3A/NFAT-luc reporter Jurkat cells were seeded at 30,000 cells/well in co-culture with CHO and EGFR-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 FcGR3A on the NFAT-luc Jurkat reporter cells and to tumor antigen EGFR on EGFR 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 EGFR-CHO cells and the Jurkat reporter cells stimulated NFAT-luciferase activity.

Activation of luciferase in FcGR3A/NFAT-Jurkat Reporter cells by Anti-FcGR3A-Anti-EGFR IgG in the presence of EGFR-CHO cells