Human Fcγ RIIIA/CD16a Antibody

Monoclonal Mouse IgG_{2A} Clone # 1001049

Catalog Number: MAB4325

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Fc gamma RIIIA/CD16a in direct ELISAs.
Source	Monoclonal Mouse IgG_{2A} Clone # 1001049
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Human embryonic kidney cell, HEK293-derived human Fc gamma RIIIA/CD16a Gly17-Gln208 Accession # P08637
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

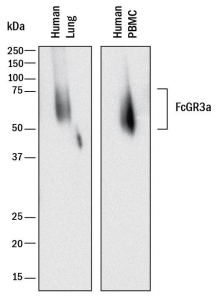
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

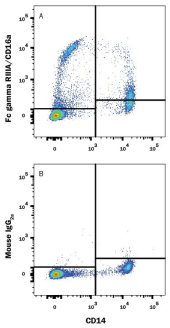
DATA

Western Blot



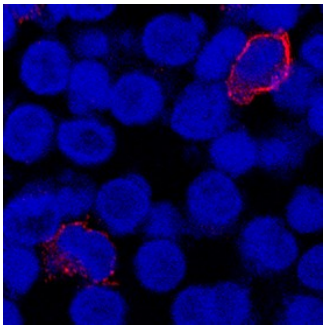
Detection of Human Fcγ RIIIA/CD16a by Western Blot. Western blot shows lysates of human lung tissue and human peripheral blood mononuclear cells (PBMCs). PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human Fcγ RIIIA/CD16a Monoclonal Antibody (Catalog # MAB4325) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Fcγ RIIIA/CD16a at approximately 50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



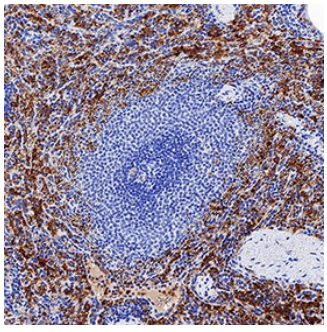
Detection of Fcγ RIIIA/CD16a in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were stained with (A) Mouse Anti-Human Fcγ RIIIA/CD16a Monoclonal Antibody (Catalog # MAB4325) or (B) Mouse IgG2A isotype control antibody (Catalog # MAB003) followed by Goat anti-Mouse IgG APC-conjugated Secondary Antibody (Catalog # F0101B) and Mouse anti-Human CD14 PE-conjugated Monoclonal Antibody (Catalog # FAB3832P). View our protocol for Staining Membrane-associated Proteins.

Immunocytochemistry



Fcγ RIIIA/CD16a in Human PBMCs. Fcγ RIIIA/CD16a was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Mouse Anti-Human Fcγ RIIIA/CD16a Monoclonal Antibody (Catalog # MAB4325) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry



Fcγ RIIIA/CD16a in Human Spleen. Fcγ RIIIA/CD16a was detected in immersion fixed paraffin-embedded sections of human spleen using Mouse Anti-Human Fcγ RIIIA/CD16a Monoclonal Antibody (Catalog # MAB4325) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and cell surfaces in lymphocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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Human Fcγ RIIIA/CD16a Antibody

Monoclonal Mouse IgG_{2A} Clone # 1001049

Catalog Number: MAB4325

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Fcγ RIIIa is a low/intermediate affinity receptor for polyvalent immune-complexed IgG. It is involved in phagocytosis, secretion of enzymes and inflammatory mediators, antibody-dependent cytotoxicity and clearance of immune complexes (1, 2). In humans, it is a 50-70 kDa type I transmembrane activating receptor expressed by NK cells, T cells, monocytes, and macrophages (1). Fcγ RIIIb is highly related, sharing 97% amino acid (aa) identity within the extracellular domain (ECD), but is a GPI-linked receptor expressed on human neutrophils and eosinophils (1, 2). The ECD of Fcγ RIIIa shares 63%, 61%, 65%, 59% and 58% aa identity with mouse Fcγ RIV, rat Fcγ RIIIa, feline CD16, bovine CD16 and porcine Fcγ RIIIb paralogs, respectively. The Fcγ RIIIa cDNA encodes 254 aa including a 16 aa signal sequence, 191 aa ECD with two C2-type Ig-like domains and five potential N-glycosylation sites, a 22 aa transmembrane (TM) sequence and a 25 aa cytoplasmic domain. In humans, a single nucleotide polymorphism creates high binding (176V) and low binding (176F) forms that, when homozygous, may influence susceptibility to autoimmune diseases or response to therapeutic IgG antibodies (3, 4). Catalog # 4325-FC is expressed as the 176V isoform of Fcγ RIIIa. Fcγ RIIIa surface expression requires interaction of an accessory chain, either the common γ-chain or CD3ζ (5, 6). Glycosylation patterns, electrophoretic mobility and binding affinity appear to differ between NK cell and monocyte Fcγ RIIIa (7). The ECD of both Fcγ RIIIa and b can be proteolytically cleaved and retain binding activity in soluble form (8-11). In monocytes and macrophages, activation and phagocytosis can trigger Fcγ RIIIa release (11). Soluble Fcγ RIII can be detected in normal plasma and is increased in rheumatoid arthritis and in coronary artery diseases (9, 10).

References:

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