

DESCRIPTION

Species Reactivity	Human
Specificity	Detects a synthetic peptide specific for human FCγR3A around amino acid 55 in Direct ELISA.
Source	Monoclonal Mouse IgG ₁ Clone # 1105901
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Synthetic Peptide Accession # P08637
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

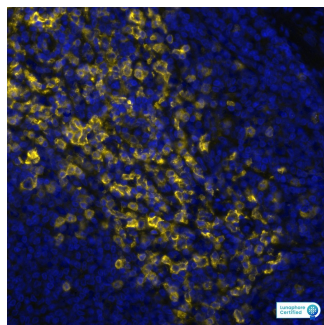
APPLICATIONS

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	1 µg/mL	Human lung tissue and human spleen tissue
Multiplex Immunofluorescence	5 µg/mL	Immersion fixed paraffin-embedded sections of human spleen
Immunohistochemistry	0.5-15 µg/mL	Immersion fixed paraffin-embedded sections of human liver and spleen

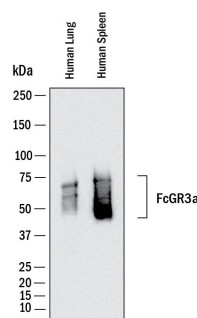
DATA

Multiplex Immunofluorescence



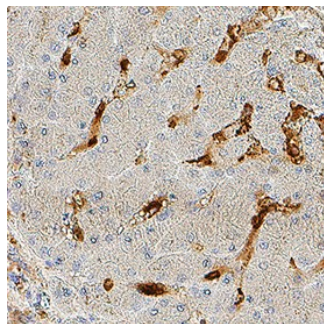
Detection of CD16a in Human Spleen via seqIF™ staining on COMET™ CD16a was detected in immersion fixed paraffin-embedded sections of human Spleen using Mouse Anti-Human CD16a, Monoclonal Antibody (Catalog # MAB11728) at 5μg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; EpreDia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37° Celsius for 4 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in [COMET™ Panel Builder](#).

Western Blot



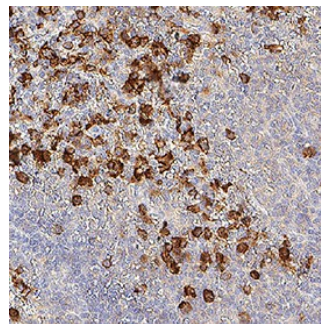
Detection of Human Fcγ RIIIA/CD16a by Western Blot. Western Blot shows lysates of human lung tissue and human spleen tissue. PVDF membrane was probed with 1 μg/ml of Mouse Anti-Human Fcγ RIIIA/CD16a Monoclonal Antibody (Catalog # MAB11728) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Fcγ RIIIA/CD16a at approximately 50-75 kDa (as indicated). This experiment was conducted under reducing conditions and using [Western Blot Buffer Group 1](#).

Immunohistochemistry



Detection of Fcγ RIIIA/CD16a in Human Liver. Fcγ RIIIA/CD16a was detected in immersion fixed paraffin-embedded sections of human liver using Mouse Anti-Human Fcγ RIIIA/CD16a Monoclonal Antibody (Catalog # MAB11728) at 1 μg/ml for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001) or the HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cell surface. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Immunohistochemistry



Detection of 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 # MAB11728) at 1 μg/ml for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001) or the HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cell surface. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 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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