Mouse Fcγ RIIIB/CD16b Antibody

Recombinant Monoclonal Rabbit IgG Clone # 3163D
Catalog Number: MAB11667

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects recombinant mouse CD16 protein in Direct ELISA.
Source	Recombinant Monoclonal Rabbit IgG Clone # 3163D
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line, NS0-derived mouse Fc gamma RIII (CD16) Ala31-Thr215 Accession # P08508
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

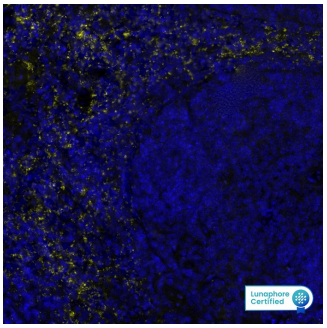
APPLICATIONS

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	2 µg/mL	Mouse spleen tissue
Immunocytochemistry	1-10 µg/mL	Immersion fixed RAW264.7 mouse monocyte/macrophage cell line and NIH-3T3 mouse embryonic fibroblast cell line
Multiplex Immunofluorescence	25 µg/mL	Immersion fixed paraffin-embedded sections of mouse spleen
Immunohistochemistry	0.1-10 µg/mL	Immersion fixed paraffin-embedded sections of mouse thymus, mouse liver and mouse lung

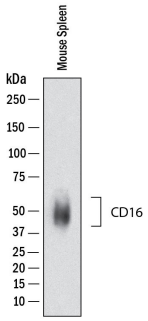
DATA

Multiplex Immunofluorescence



Detection of CD16 in Mouse Spleen via seqIF™ staining on COMET™ CD16 was detected in immersion fixed paraffin-embedded sections of mouse Spleen using Mouse Anti-Mouse CD16, Monoclonal Antibody (Catalog #MAB11667) at 25ug/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-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the cytoplasm and membrane. Protocol available in COMET™ Panel Builder.

Western Blot



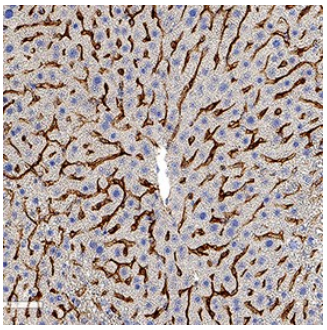
Detection of Mouse Fcγ RIIIB/CD16b by Western Blot. Western Blot shows lysates of mouse spleen tissue. PVDF membrane was probed with 2 µg/ml of Rabbit Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11667) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Fcγ RIIIB/CD16b at approximately 40-60 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

Mouse Fcγ RIIIB/CD16b Antibody

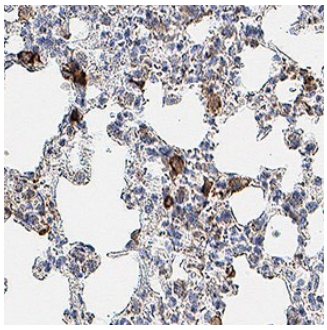
Recombinant Monoclonal Rabbit IgG Clone # 3163D
Catalog Number: MAB11667

Immunohistochemistry



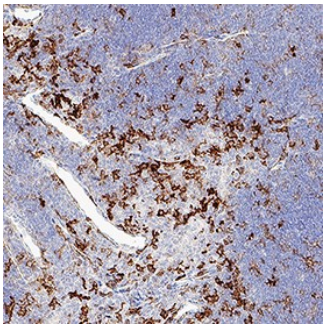
Detection of Fcγ RIIIB/CD16b in Mouse Liver.
Fcγ RIIIB/CD16b was detected in immersion fixed paraffin-embedded sections of mouse liver using Rabbit Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11667) at 0.1 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). 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 of Kupffer cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



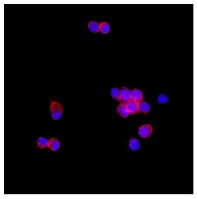
Detection of Fcγ RIIIB/CD16b in Mouse Lung.
Fcγ RIIIB/CD16b was detected in immersion fixed paraffin-embedded sections of mouse lung using Rabbit Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11667) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). 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 of macrophages. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry

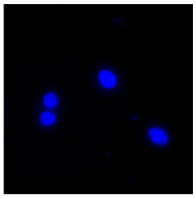


Detection of Fcγ RIIIB/CD16b in Mouse Thymus.
Fcγ RIIIB/CD16b was detected in immersion fixed paraffin-embedded sections of mouse thymus using Rabbit Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11667) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). 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.

Immunocytochemistry



Positive (RAW264.7 cells)



Negative (NIH-3T3 cells)

Detection of Fcγ RIIIB/CD16b in RAW264.7 cells
Fcγ RIIIB/CD16b was detected in immersion fixed RAW264.7 mouse monocyte/macrophage cell line (Positive) and absent in NIH-3T3 mouse embryonic fibroblast cell line (Negative) using Rabbit Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11667) at 3 µg/ml for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to the cell surface of RAW264.7 (positive) cells. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
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.

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

Mouse Fcγ RIIIB/CD16b Antibody

Recombinant Monoclonal Rabbit IgG Clone # 3163D
Catalog Number: MAB11667

BACKGROUND

Receptors for the Fc region of IgG (Fc γ Rs) are members of the Ig superfamily that function in the activation or inhibition of immune responses such as degranulation, phagocytosis, ADCC (antibody-dependent cellular toxicity), cytokine release, and B cell proliferation (1-3). The Fc γ Rs have been divided into three classes based on close relationships in their extracellular domains; these groups are designated Fc γ RI (also known as CD64), Fc γ RII (CD32), and Fc γ RIII (CD16). Each group may be encoded by multiple genes and exist in different isoforms depending on species and cell type. The CD64 proteins are high affinity receptors (~10^{-8}-10^{-9} M) capable of binding monomeric IgG, whereas the CD16 and CD32 proteins bind IgG with lower affinities (~10^{-6}-10^{-7} M) only recognizing IgG aggregates surrounding multivalent antigens (1, 4). Fc γ Rs that deliver an activating signal either have an intrinsic immunoreceptor tyrosine-based activation motif (ITAM) within their cytoplasmic domains or associate with one of the ITAM-bearing adapter subunits, Fc Rγ or ζ (3, 5). The only inhibitory member in human and mouse, Fc γ RIIb, has an intrinsic cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM). The coordinated functioning of activating and inhibitory receptors is necessary for successful initiation, amplification, and termination of immune responses (5).

Mouse CD16 is encoded by a single gene. The protein product is a type I transmembrane protein having two extracellular Ig-like domains. It is expressed on a variety of myeloid and lymphoid cells (4) and associates with Fc Rγ to deliver an activating signal upon ligand binding (5). Mouse CD32 is closely related to mouse CD16 throughout its extracellular domain (95% amino acid sequence identity), but has a divergent cytoplasmic domain and functions as an inhibitory receptor. Together these proteins constitute an activating/inhibiting receptor pair to regulate immune responses (5).

References:

1. van de Winkel, J. and P. Capes (1993) Immunol. Today **14**:215.
2. Raghaven, M. and P. Bjorkman (1996) Annu. Rev. Cell Dev. Biol. **12**:181.
3. Ravetch, J. and S. Bolland (2001) Annu. Rev. Immunol. **19**:275.
4. Takai, T. (2002) Nature Rev. Immunol. **2**:580.
5. Ravetch, J. and L. Lanier (2000) Science **290**:84.

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475