

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects a synthetic peptide specific for mouse CD16b around amino acid 60 in Direct ELISA.
Source	Monoclonal Rat IgG _{2B} Clone # 1107512
Purification	Protein A or G purified from hybridoma culture supernatant
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

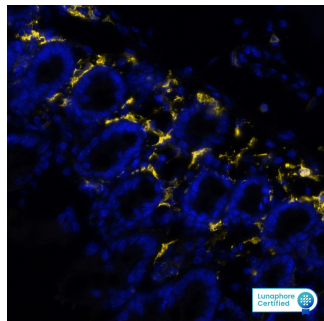
APPLICATIONS

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 liver tissue
Multiplex Immunofluorescence	10 µg/mL	Perfusion fixed paraffin-embedded sections of Mouse Colon, Spleen, Thymus and Stomach
Immunohistochemistry	3-25 µg/mL	Perfusion fixed paraffin-embedded sections of mouse liver and thymus

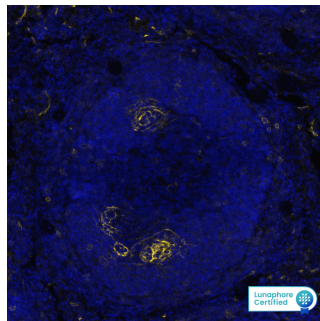
DATA

Multiplex Immunofluorescence



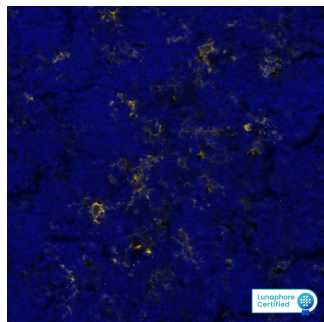
Detection of CD16b in Mouse Colon via seqIF™ staining on COMET™ CD16b was detected in perfusion fixed paraffin-embedded sections of mouse Colon using Rat Anti-Mouse CD16b, Monoclonal Antibody (Catalog #MAB11725) at 10ug/mL at 37° Celsius for 4 minutes. Before incubation with the primary antibody, tissue 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™ 555 Goat anti-Rat IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # [DR555RT](#)) and counterstained with DAPI (blue; Lunaphore Catalog # [DR100](#)). Specific staining was localized to the cytoplasm. Protocol available in [COMET™ Panel Builder](#).

Multiplex Immunofluorescence



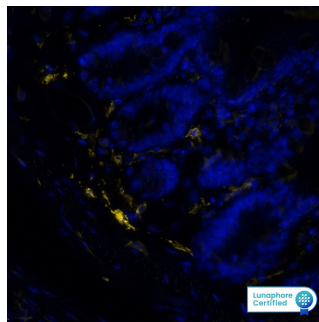
Detection of CD16b in Mouse Spleen via seqIF™ staining on COMET™ CD16b was detected in perfusion fixed paraffin-embedded sections of mouse Spleen using Rat Anti-Mouse CD16b, Monoclonal Antibody (Catalog #MAB11725) at 10ug/mL at 37° Celsius for 4 minutes. Before incubation with the primary antibody, tissue 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™ 555 Goat anti-Rat IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # [DR555RT](#)) and counterstained with DAPI (blue; Lunaphore Catalog # [DR100](#)). Specific staining was localized to the cytoplasm. Protocol available in [COMET™ Panel Builder](#).

Multiplex Immunofluorescence



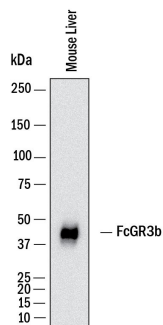
CD16b Antibody in Mouse Thymus via seqIF™ staining on COMET™ CD16b was detected in perfusion fixed paraffin-embedded sections of mouse Thymus using Rat Anti-Mouse CD16b, Monoclonal Antibody (Catalog #MAB11725) at 10ug/mL at 37° Celsius for 4 minutes. Before incubation with the primary antibody, tissue 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™ 555 Goat anti-Rat IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # [DR555RT](#)) and counterstained with DAPI (blue; Lunaphore Catalog # [DR100](#)). Specific staining was localized to the cytoplasm. Protocol available in [COMET™ Panel Builder](#).

Multiplex Immunofluorescence



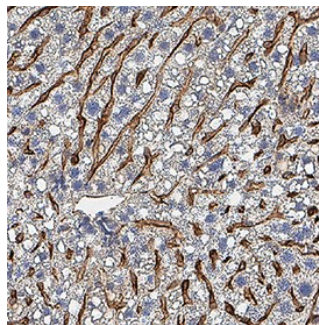
Detection of CD16b in Mouse Stomach via seqIF™ staining on COMET™ CD16b was detected in perfusion fixed paraffin-embedded sections of mouse Stomach using Rat Anti-Mouse CD16b, Monoclonal Antibody (Catalog #MAB11725) at 10ug/mL at 37° Celsius for 4 minutes. Before incubation with the primary antibody, 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™ 555 Goat anti-Rat IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # [DR555RT](#)) and counterstained with DAPI (blue; Lunaphore Catalog # [DR100](#)). Specific staining was localized to the cytoplasm. Protocol available in [COMET™ Panel Builder](#).

Western Blot



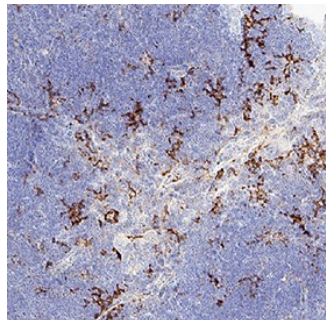
Detection of Mouse Fcγ RIIIB/CD16b by Western Blot. Western Blot shows lysates of mouse liver tissue. PVDF membrane was probed with 2 µg/ml of Rat Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11725) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # [HAF005](#)). A specific band was detected for Fcγ RIIIB/CD16b at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using [Western Blot Buffer Group 1](#).

Immunohistochemistry



Detection of Fcγ RIIIB/CD16b in Mouse Liver. Fcγ RIIIB/CD16b was detected in perfusion fixed paraffin-embedded sections of mouse liver using Rat Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11725) at 5 µg/ml overnight at 4 °C. 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 the HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # [HAF005](#)) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. 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 perfusion fixed paraffin-embedded sections of mouse thymus using Rat Anti-Mouse Fcγ RIIIB/CD16b Monoclonal Antibody (Catalog # MAB11725) at 5 µg/ml overnight at 4 °C. 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 the HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. 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	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.

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 γ 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 γ 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.