Human CXCR7/RDC-1 Antibody

Monoclonal Mouse IgG_{2A} Clone # 358426

Catalog Number: MAB4227

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CXCR7/RDC-1 in direct ELISAs. In flow cytometry, reacts specifically with five distinct human CXCR7 transfectants, but does not react with their respective parental lines or mouse CXCR7 transfectants. In flow cytometry, also reacts with monocytes expressing CXCR7, but does not react with MCF-7 cells which have been reported to have surface-expressing CXCR7 using clone 11G8. Due to the conflicting reports published, use of monoclonal MAB4227 may result in an underestimation of CXCR7 expression on certain cell types.
Source	Monoclonal Mouse IgG_{2A} Clone # 358426
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	NS0 mouse myeloma cell line transfected with human CXCR7/RDC-1 Met1-Lys362 (Gly131Ser) Accession # AAA62370
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.

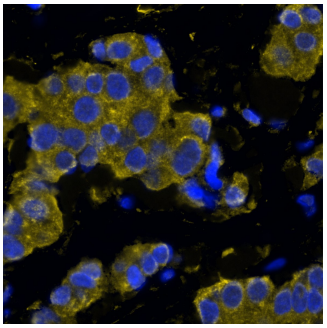
APPLICATIONS

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
Flow Cytometry	2.5 µg/10^6 cells	See Below
Multiplex Immunofluorescence	15 µg/mL	Immersion fixed paraffin-embedded sections of Human Breast Cancer tissue
Immunohistochemistry	8-25 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.	

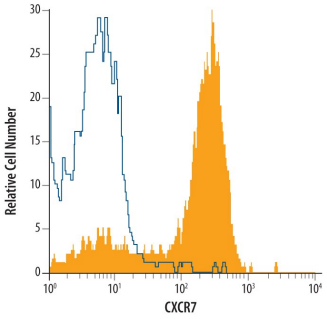
DATA

Multiplex Immunofluorescence



Detection of CXCR7 in Human Breast Tumor via Multiplex Immunofluorescence staining on COMET™ CXCR7 was detected in immersion fixed paraffin-embedded sections of human breast tumor using Mouse Anti-Human CXCR7 Monoclonal Antibody (Catalog # Catalog # MAB4227) at 15ug/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). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the cytoplasm. Protocol available in COMET™ Panel Builder.

Flow Cytometry



Detection of CXCR7/RDC-1 in Human peripheral blood Monocytes by Flow Cytometry. Human peripheral blood monocytes were stained with Mouse Anti-Human CXCR7/RDC-1 Monoclonal Antibody (Catalog # MAB4227, filled histogram) or isotype control antibody (Catalog # Catalog # MAB003, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG $F(ab')_2$Secondary Antibody (Catalog # Catalog # F0101B).

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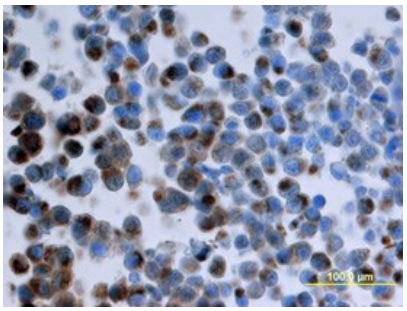
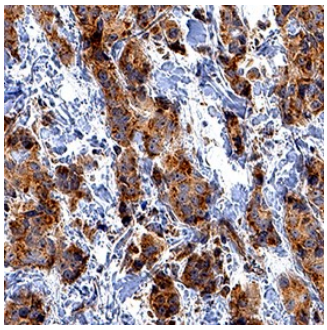
Immunohistochemistry



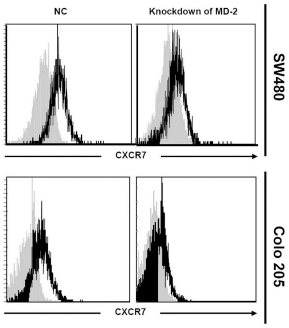
CXCR7/RDC-1 in Human Breast Cancer Tissue. CXCR7/RDC-1 was detected in perfusion fixed paraffin-embedded sections of nude mice injected with human breast cancer cells using Mouse Anti-Human CXCR7/RDC-1 Monoclonal Antibody (Catalog # MAB4227) at 25 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS002) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunohistochemistry



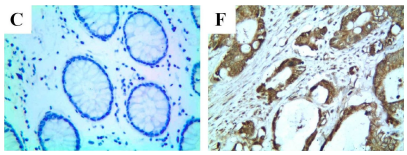
CXCR7/RDC-1 in Human Breast Cancer Tissue. CXCR7/RDC-1 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Mouse Anti-Human CXCR7/RDC-1 Monoclonal Antibody (Catalog # MAB4227) at 0.5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and plasma membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Flow Cytometry



Detection of Human CXCR7/RDC-1 by Flow Cytometry Knockdown effect of MD-2 on exposure of TLR4 to LPS in SW480 and Colo 205 cell lines.A, SW480 and Colo 205 cell lines were transfected transiently with siRNA or negative control sequence(NC). SW480 and Colo 205 cell lines transfected with the MD-2 siRNA sequence exhibited a marked reduction in MD-2 mRNA and protein level compared with NC. B, After LPS treatment, flow cytometry and real-time quantitative-PCR were performed. Knockdown of MD-2 inhibited LPS-mediated CXCR7 expression. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/22180778), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunocytochemistry/ Immunofluorescence



Detection of Human CXCR7/RDC-1 by Immunocytochemistry/Immunofluorescence Representative examples of immunohistochemical staining of TLR4, MD-2, and CXCR7 in colorectal carcinoma tissues (original magnification 100×).Positive staining was observed as a dark brown color. Normal colorectal tissues showed negative immunohistochemical staining of TLR4 (A), MD-2 (B), and CXCR7 (C), and colorectal carcinoma tissues showed strong staining of TLR4 (D), MD-2 (E), and CXCR7 (F). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/22180778), licensed under a CC-BY license. Not internally tested by R&D Systems.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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	**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.

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BACKGROUND

The G protein-coupled receptor, RDC1, belongs to a subgroup of chemokine receptors and has been designated CXCR7. CXCR7 can bind with high-affinity to CXCL12/SDF-1 and CXCL11/I-TAC. It is also a co-receptor for several HIV and SIV strains. In their N-termini and extracellular loops 1, 2, and 3, human and mouse CXCR7 share 84%, 100%, 96% and 86% amino acid sequence identity, respectively. Reports of mRNA levels and/or protein expression (as assessed using anti-CXCR7, clone 9C4) (1, 2) indicate that CXCR7 occurs on a wide variety of tissues and cells including monocytes, B cells, T cells and mature dendritic cells. In contrast, based on ligand binding analysis and receptor level (as assessed using anti-CXCR7, clone 11G8), surface expression of CXCR7 was reported to be restricted to tumor cells, activated endothelial cells, fetal liver cells, and few other cell types (3). The basis of these inconsistent observations is not known but may be attributed to cell context and the use of different antibodies that may recognize different epitopes.

References:

1. Balabanian, K. *et al.* (2005) J. Biol. Chem. **280**:35760.
2. Infantino, S. *et al.* (2006) J. Immunol. **176**:2197.
3. Burns, J.M. *et al.* (2006) J. Exp. Med. **203**:2201.

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