Product Information

Monoclonal Anti-CXCR-2 (IL-8 RB), Clone 48311

Produced in mouse, Purified immunoglobulin

C6348

Product Description

Monoclonal Anti-Human CXCR-2 (IL-8 RB) (mouse IgG2a isotype) is derived from the 48311.211 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a Balb/c mouse immunized with human CXCR-2 transfected NSO mouse myeloma cells. The antibody is purified from ascites fluid using protein G chromatography.

Monoclonal Anti-Human CXCR-2 reacts with CXCR-2 transfected cells and not with the parent cell line by flow cytometry. The antibody shows no cross-myeloperoxidase reactivity with human CXCR-1. The antibody will neutralize human cell surface CXCR-2 mediated-myeloperoxidase release from human granulocytes induced by GROα. It will not block myeloperoxidase release induced by IL-8.

Monoclonal Anti-Human CXCR-2 may be used to detect CXCR-2 present on human blood cells by flow cytometry. The antibody may be used to neutralize human cell surface CXCR-2 mediated bioactivity.

Chemokines have been sub-divided into families based on the relative position of their cysteine residues. The α- and β-families, with four cysteine residues, are the largest and best characterized. In the α-family, one amino acid separates the first two cysteine residues (CXC); in the β-family the two cysteine residues (CC) are adjacent to each other. The α-chemokines that contain the N-terminal Glu-Leu-Arg amino acid sequence (ELR-motif) are chemotactic for neutrophils (such as IL-8), while those that do not act on lymphocytes (such as IP-10 and MIG). Examples of chemokines under the β-family category are MCP1-5 and RANTES. The chemokine lymphotactin belongs to the γ-family, with only two cysteines (C), and the recently described fractalkine, or neurotactin, is a member of the *-family and has the first two cysteine residues separated by three amino-acids (CXXXC).

Chemokines bind to specific G protein-coupled cell surface receptors on target cells. Five CXC receptors (CXCR1-5), nine CC receptors (CCR1-9) and one CXXXC receptor (CX_3CR1) have been cloned to date. Expression of chemokine receptors can be restricted to some cell types (CXCR1 is expressed in neutrophils) while others (such as CCR2) are expressed in a wide variety of cells.[1] Receptor expression has also been found to be constitutive (including down regulation), inducible or restricted to a cell state of activation. In addition, some chemokine receptors are also expressed in non-hematopoietic cells, such as nerve, endothelial and epithelial cells. This suggests that chemokines have other roles besides leucocyte chemotaxis. CX_3CR1, for example, is highly expressed in adult brain.

Chemokine receptors are linked to phospholipases through the Gi class of G proteins (inhibition by pertussis toxin). Receptor activation leads to a cascade of cellular events including generation of inositol triphosphate, calcium release and activation of protein kinase C. Chemokine receptors also activate small GTP-binding proteins of the Ras and Rho families, the latter being involved in cell motility events. In addition, chemokines bind to non-signaling molecules such as the Duffy antigen receptor for chemokines (DARC) which may act to remove chemokines from the circulation, and heparan sulfates proteoglycans which may serve to establish an ECM concentration gradient.

CXCR-1 (IL-8RA, or type I IL-8 receptor) and CXCR-2 (IL-8RB, or type II IL-8 receptor) have been shown to share ~77% amino acid sequence identity. IL-8 binds to both receptors with high affinity and induces rapid elevation of cytosolic Ca^{2+} levels.[2-4] Whereas CXCR-1 is highly specific for IL-8, CXCR-2 has broad specificity and has been shown to bind with high-affinity to other ELR motif containing a chemokine including GROα, GROβ, GROγ, NAP-2, and ENA-78. In contrast, PF4 and IP-10 (two chemokines that lack the ELR motif) have been shown to lack binding affinity for CXCR-2. CXCR-1 and CXCR-2 are expressed by neutrophils but not B lymphocytes or T lymphocytes.

Reagent

Lyophilized from 0.2 µm-filtered solution in PBS, pH 7.4 with 5% Trehalose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 µm-filtered phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at −20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at −20 °C. Avoid repeated freezing and thawing.

Procedure

Monoclonal Anti-CXCR-2 is tested for its ability to block human cell surface CXCR-2 mediated bioactivity of recombinant human GROα in a myeloperoxidase release assay using human granulocytes.[5] The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of the cell surface CXCR-2 mediated GROα response on a responsive cell line.

Product Profile

For neutralization, a working concentration of 0.5-1.5 µg/mL of antibody will block 50% of the bioactivity due to 1 µg/mL recombinant human GROα in an assay measuring myeloperoxidase released from human granulocytes.

For flow cytometry, the use of 10 µL at 0.25 µg/10^6 cells is recommended. The volume of cell suspension can be variable. The volume of cell suspension must not exceed 200 µL.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration.

Endotoxin level is <0.10 EU per 1 µg antibody as determined by the LAL method.

References

1. Wells, N.C., et al., Trends Pharm. Sci., 19, 376 (1998).
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3. Lee, J., et al., J. Biol. Chem., 267, 16283 (1992).
4. Holmes, W.E., et al., Science, 253, 1278 (1991).
5. Schröder, J., et al., J. Immunol., 139, 3474 (1987).

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