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ProductInformation

Monoclonal Anti-CXCR-1 (IL-8 RA)
Clone 42705.111
Purified Mouse Immunoglobulin

Product Number **C6223**

Product Description

Monoclonal Anti-Human CXCR-1 (IL-8 RA) (mouse IgG2a isotype) is derived from the 42705.111 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a Balb/c mouse immunized with human CXCR-1 transfected NSO mouse myeloma cells. The antibody is purified from ascites fluid using protein G chromatography.

Monoclonal Anti-Human CXCR-1 reacts with CXCR-1 transfected cells and not with the parent cell line by flow cytometry. The antibody shows no cross-reactivity with human CXCR-2. The antibody may be used in various techniques including neutralization, flow cytometry, and immunhistochemistry (frozen sections).

Monoclonal Anti-Human CXCR-1 may be used detect CXCR-1 present on human blood cells by flow cytometry. The antibody may be used to neutralize human cell surface CXCR-1 mediated bioactivity.

Chemokines have been sub-divided into families on the basis of 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 δ-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 approximately 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 chemokines 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
The antibody is supplied lyophilized from a 0.2 µm filtered solution in phosphate buffered saline with 5% trehalose.

Preparation Instructions
To one vial of lyophilized powder, add 1 mL of 0.2 µm-filtered PBS 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.

Product Profile
For neutralization, the antibody will neutralize recombinant human IL-8-induced chemotactic effect on BaF/3 hCXCR-1 cells.

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-1 mediated IL-8 response on a responsive cell line.

For flow cytometry, a working antibody concentration of 10 µg/mL is determined using 10 µL of diluted antibody and human blood cells (10^5-10^6).

For immunohistochemistry, a working antibody concentration of 25 µg/mL is recommended using frozen human tissue sections (spleen) and either fluorescent probes or chromogenic staining reagents.

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

References

1. Wells, N.C., et al., Trends Pharm. Sci., **19**, 376 (1998).
2. Murphy, R.P., and Tiffany, H.L., Science, 253, 1280 (1991).
3. Lee, J., et al., J. Biol. Chem., 267, 16283 (1992).
4. Holmes, W.E., et al., Science, 253, 1278 (1991).

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