

Product Information

Monoclonal Anti-CCR-5

Clone 45523

produced in mouse, purified immunoglobulin

Catalog Number **C5973**

Product Description

Monoclonal Anti-CCR-5 (mouse IgG2b isotype) is derived from the 45523 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a Balb/c mouse immunized with human CCR-5 transfected NSO mouse myeloma cells. The antibody is purified from ascites fluid using protein G chromatography.

Monoclonal Anti-CCR-5 recognizes human CCR-5. The antibody may be used in various immunochemical techniques including flow cytometry and immunohistochemistry. Monoclonal Anti-CCR-5 reacts with human CCR-5 transfected cells and not the parent cell line by flow cytometry. The antibody shows no cross-reactivity with CCR-1, CCR-2, or CCR-3. It detects CCR-5 present on stimulated human PBMCs (peripheral blood mononuclear cells) only after the cells are fixed with 2% formaldehyde. The antibody does not detect CCR-5 on unfixed human PBMCs.

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). 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 (CX₃CR1) 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. CX₃CR1, 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.

CCR-5 has 48-75% amino acid sequence identity to earlier identified CC receptors.^{2,3} It is expressed in primary adherent monocytes, but not in neutrophils or eosinophils.⁴⁻⁸ CCR-5 mediates the activities of MIP-1a, MIP-1b, and RANTES. Recently, it has also been shown to be a co-receptor on CD4+ target cells for infection with primary, monocyte-tropic HIV-1 viruses.⁹⁻¹¹

Reagent

Supplied lyophilized from a 0.2 μ m filtered solution of 5% trehalose in phosphate buffered saline.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 μ m-filtered PBS to produce a 0.5 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be necessary.

Precautions and Disclaimer

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

Storage/Stability

Prior to reconstitution, store at -20°C . Reconstituted product may be stored at $2-8^{\circ}\text{C}$ for at least one month. For extended storage of the reconstituted solution, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers is not recommended.

Product Profile

Flow Cytometry: dilute this antibody to $25\ \mu\text{g}/\text{mL}$ and add $10\ \mu\text{L}$ of this dilution to 1 to 2.5×10^5 cells in a total reaction volume not exceeding $200\ \mu\text{L}$.

Immunohistochemistry: a working concentration of $25\ \mu\text{g}/\text{mL}$ is recommended using paraffin-embedded human lymph node tissue sections.

Endotoxin level is < 0.1 endotoxin unit/ μg antibody as determined by the LAL method.

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

References

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