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Product Information

Monoclonal Anti-Human CCR-2

Clone 48607.121

Purified Mouse Immunoglobulin

Product Number **C 5848**

Product Description

Monoclonal Anti-Human CCR-2 (mouse IgG2b isotype) is derived from the 48607.121 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a Balb/c mouse immunized with human CCR-2 transfected NSO mouse myeloma cells. The antibody is purified from ascites fluid using protein G chromatography.

Monoclonal Anti-Human CCR-2 reacts with CCR-2 transfected cells and not the parent cell line by flow cytometry. The antibody shows no cross-reactivity with CCR-5 transfected cells.

Monoclonal Anti-Human CCR-2 may be used the identification of human CCR-2 by flow cytometry.

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 (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-2A and CCR-2B (MCP-1RA and MCP-1RB) differ in their alternatively spliced carboxy-terminus and are probably spliced variants of a single gene.¹ CCR-2A and B specifically bind MCP-1 and MCP-3. The two receptors are expressed on monocytes but not on neutrophils or eosinophils.

Reagent

The antibody is supplied lyophilized from a 0.2 μm filtered solution in phosphate buffered saline containing 5% trehalose.

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

Store at $-20\text{ }^{\circ}\text{C}$. Reconstituted product may be stored at $2-8\text{ }^{\circ}\text{C}$. for up to one month. For prolonged storage, freeze in working aliquots. Avoid repeated freezing and thawing.

Product Profile

Working concentration is 5-10 $\mu\text{g}/\text{ml}$ as determined by flow cytometry using $10\text{ }\mu\text{l}$ (10^5) human CCR-2 transfected cells.

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

Endotoxin level is $< 10\text{ ng}$ per mg antibody as determined by the LAL method.

References

1. Wells, N. C., et al., Trends Pharm. Sci., **19**, 376 (1998).
2. Charo, I. F., et al., Proc. Natl. Acad. Sci. USA, **91**, 2752 (1994).

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