



## Product Information

### MONOCLONAL ANTI-HUMAN CXCR-4 (Fusin)

Clone 44716.111

Purified Mouse Immunoglobulin

Product Number **C6598**

#### Product Description

Monoclonal Anti-Human CXCR-4 (Fusin) (mouse IgG2b isotype) is derived from the 44716.111 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a Balb/c mouse immunized with human CXCR-4 transfected NSO mouse myeloma cells. The antibody is purified from ascites fluid using protein G chromatography.

Monoclonal Anti-Human CXCR-4 reacts with CXCR-4 (fusin) transfected cells and not with the parent cell line by flow cytometry. The antibody also reacts with cells expressing feline CXCR-4, but not rat CXCR-4. The antibody shows no cross-reactivity with other chemokine receptors. The antibody will neutralize human cell surface CXCR-4 mediated SDF/PBSF response using human cultured lymphocytes in a chemotaxis assay.

Monoclonal Anti-Human CXCR-4 may be used detect CXCR-4 present on human cells by flow cytometry. The antibody may be used to neutralize human cell surface CXCR-4 mediated bioactivity. The antibody may also be used for immunohistology and immunofluorescence. Chemokines have been sub-divided into families on the basis of the relative position of their cysteine residues. The  $\alpha$ - and  $\beta$ - families, with four cysteine residues, are the largest and best characterized. In the  $\alpha$ -family, one amino acid separates the first two cysteine residues (CXC); in the  $\beta$ -family the two cysteine residues (CC) are adjacent to each other. The  $\alpha$ -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  $\beta$ -family category are MCP1-5 and RANTES. The chemokine lymphotactin belongs to the  $\gamma$ -family, with only two cysteines (C), and the recently described fractalkine or neurotactin is a member of the  $\delta$ -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<sub>3</sub>CR1) 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.<sup>1</sup> 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<sub>3</sub>CR1, for example, is highly expressed in adult brain.

Chemokine receptors are linked to phospholipases through the G<sub>i</sub> 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-4, also known as fusin or LESTR,<sup>2-3</sup> was originally discovered as an orphan receptor with structural similarity to chemokine receptors. CXCR-4 was subsequently identified as a necessary cofactor for entry of T cell-tropic HIV viruses into CD4<sup>+</sup> cells.<sup>2</sup> The CXC chemokine PBSF/SDF-1 has now been shown to be the ligand for CXCR-4 and a powerful inhibitor of infection by T cell-tropic HIV-1 strains.<sup>4-5</sup>

#### Reagents

The product is supplied lyophilized from a 0.2  $\mu$ m filtered solution in phosphate buffered saline. Endotoxin level is < 10 ng per mg antibody as determined by the LAL method.

### Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2  $\mu$ m-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  $-20\text{ }^{\circ}\text{C}$ . Reconstituted product may be stored at  $2-8\text{ }^{\circ}\text{C}$  for at least one month. For prolonged storage, freeze in working aliquots at  $-20\text{ }^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

### Product Profile

For neutralization, a working concentration of 2.5-20  $\mu$ g/ml of monoclonal anti-CXCR-4 will block 50% of the bioactivity due to 0.1  $\mu$ g/ml recombinant human SDF-1 in an assay measuring chemotaxis using BaF/3 human CXCR-4 transfected cells.

The  $\text{ND}_{50}$  of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of the cell surface CXCR-4 mediated SDF-1/PBSF response on a responsive cell line.

For flow cytometry, a working concentration of 10  $\mu$ g/ml is determined using  $10\text{ }\mu\text{l}$  ( $10^5$ - $10^6$ ) cells.<sup>6</sup>

For immunofluorescence, a working concentration of 5  $\mu$ g/ml is determined using stimulated human peripheral blood mononuclear cells (PBMC) fixed with 2% formaldehyde and permeabilized with 0.1% saponin.

For immunohistochemistry, a working concentration of 0.5 - 5  $\mu$ g/ml is determined using human CXCR-4 cultured cells or tissue sections.

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

### References

1. Wells, N.C., et al., Trends Pharm. Sci., **19**, 376 (1998).
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6. Endres, M.J., et al., Cell, **87**, 745 (1996).

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