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ProductInformation

MONOCLONAL ANTI-EOTAXIN, MOUSE
CLONE 42285.111
Purified Rat Immunoglobulin

Product Number **E 4150**

Product Description

Monoclonal Anti-Eotaxin (rat IgG2A) is developed in rat using purified recombinant mouse eotaxin, expressed in *E. coli* as immunogen. The antibody is purified from the IgG fraction of the tissue culture supernatant using protein G affinity chromatography.

Monoclonal Anti-Eotaxin recognizes recombinant mouse eotaxin by various immunochemical techniques including immunoblotting, ELISA, and neutralization. From ELISA and immunoblotting, this antibody exhibits less than 10 % cross-reactivity with recombinant human eotaxin, recombinant mouse MARC, and recombinant human 6Ckine.

Eotaxin, a member of the beta (CC) chemokine family of inflammatory and immunoregulatory cytokines, was originally purified from bronchoalveolar lavage fluid of guinea pigs sensitized by aerosol challenge with ovalbumin. Eotaxin has been found in three species (human, mouse, and guinea pig), which share approximately 60 % sequence homology.[1] Mouse eotaxin also shows high sequence identity to the MCP family of cytokines. Mouse eotaxin cDNA [2] encodes a 97 amino acid residue precursor protein from which the amino-terminal 23 amino acid residues are cleaved to generate the 74 amino acid residue mature mouse eotaxin. Mature mouse eotaxin has a predicted molecular mass of approximately 8.4 kDa.

Eotaxin is a potent eosinophil activator and chemo-attractor. As an activator, eotaxin is a mediator of inflammation by initiating actin polymerization intra-cellularly and inducing the production of reactive oxygen species.[3] As a chemoattractant both *in vivo* and *in vitro*, eotaxin exhibits potent activity on eosinophils, but not mononuclear cells or neutrophils.[3, 4, 5] Eotaxin has the ability to prime eosinophils for chemotaxis, direct their migration, and to activate inflammatory activity. Eotaxin mRNA is expressed in a variety of tissues. Its expression is induced in cultured endothelial cells in response to IFN-γ. Eotaxin mRNA is also induced in response to the transplantation of IL-4-secreting tumor cells.

A receptor for human eotaxin has been identified and found to be the third numbered receptor in the C-C chemokine subfamily of receptors (CCR-3).[1] The mouse counterpart to human CCR-3 has also been isolated. This receptor for mouse eotaxin binds both mouse and human eotaxin with equal affinity.[6]

Reagents

Monoclonal Anti-Eotaxin is supplied as 500 µg of antiserum lyophilized from a 0.2 µm filtered solution in phosphate buffered saline (PBS).

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate-buffered saline (PBS) to produce a 0.5 mg/ml stock solution of antibody.

Storage/Stability

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

Product Profile

Monoclonal Anti-Eotaxin has the ability to neutralize the biological activity of recombinant mouse eotaxin. It will also neutralize the biological activity of human eotaxin under similar conditions.

To measure this chemoattractant activity, recombinant mouse eotaxin is incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microplate. Following this preincubation period, 35 µl of the cytokine-antibody solution (containing recombinant mouse eotaxin at a final concentration of 0.1 µg/ml and antibody at concentrations from 0.01 to 100 µg/ml) is transferred to the lower compartment of a 96 well chemotaxis chamber. The chemotaxis chamber is then assembled using a PVP-free polycarbonate filter (5 micron pore size) and 2 x 10^6 cells/ well (CCR-3 transfected Y3 rat myeloid cells) are added to the top chamber. After incubation for 3 hours at 37 °C in a 5 % CO_2 humidified incubator, the chamber is disassembled and the cells that have migrated through to the lower

chamber are transferred to a working plate and stained using MTT. Absorbance is read on a microplate reader. The exact concentration of antibody required to neutralize recombinant mouse eotaxin activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity.

The Neutralization $Dose_{50}$ (ND_{50}) for the antibody is defined as that concentration required to yield one-half maximal inhibition of the eotaxin activity on a responsive cell line, when eotaxin is present at a concentration just high enough to elicit a maximum response.

The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is approximately 10 to 30 µg/ml in the presence of 100 ng/ml of recombinant mouse eotaxin, measuring the chemotaxis of CCR-3 transfected rat myeloid Y3 cells.

For immunoblotting, a working concentration of the antibody at 1 to 2 µg/ml is recommended. The detection limit for recombinant mouse eotaxin is approximately 20 ng/lane under non-reducing and reducing conditions.

For ELISAs, a working concentration of the antibody at 0.5 to 1.0 µg/ml is recommended. The detection limit for recombinant mouse eotaxin is approximately 1.2 ng/well.

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

Endotoxin: <10 ng/mg antibody determined by the LAL method.

References

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6. Gao, J.L., et al., Identification of a mouse eosinophil receptor for the CC chemokine eotaxin. Biochem. Biophys. Res. Commun., **223**, 679 (1996).

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