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ProductInformation

MONOCLONAL ANTI-EOTAXIN-2 (MPIF-2), HUMAN CLONE 61016.11
Purified Mouse Immunoglobulin

Product Number **E 5768**

Product Description

Monoclonal Anti-Eotaxin-2 (mouse IgG1) is developed in mouse using purified recombinant human eotaxin-2, expressed in *E. coli* as immunogen. The antibody is purified from the IgG fraction of ascites fluid using protein G affinity chromatography.

Monoclonal Anti-Eotaxin-2 (MPIF-2) recognizes recombinant human eotaxin-2 by various immuno-chemical techniques including immunoblotting, ELISA, and neutralization. When used as a capture antibody in human eotaxin-2 sandwich ELISAs, this antibody exhibits less than 0.03 % cross-reactivity with recombinant human eotaxin, MIP-1α, and MCP-3.

Eotaxin-2, also named MPIF-2, Ckβ6, and SCYA24, is a member of the CC chemokine family, based on the presence of the CC motif and homology with other known CC chemokines.[1,2] Eotaxin-2 cDNA [2] encodes a 119 amino acid residue precursor protein with a 26 amino acid residue signal peptide that is cleaved to generate a mature protein predicted to contain 93 amino acid residues with an N-glycosylation site. Mature human eotaxin-2 has a predicted molecular mass of approximately 10.6 kDa. Compared to other CC chemokines, eotaxin-2 exhibits 40 %, 42 %, and 39 % amino acid identity to MCP-3, MIP-1α, and eotaxin, respectively. Human CC chemokine eotaxin-2 maps to chromosome 7q11.23.[3]

Both eotaxin and eotaxin-2 activate and attract eosinophils and basophils. 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).[4] On eosinophils, the effects of eotaxin-2 is inhibited by an anti-CCR-3 antibody and cross-desensitized by eotaxin and MCP-4, suggesting that all three CC chemokines act through CCR-3.[2,5]

Eotaxin-2 mRNA is weakly expressed in activated monocytes and T lymphocytes. Recombinant eotaxin-2 induces chemotaxis of eosinophils, basophils, and resting T lymphocytes but not monocytes and activated T lymphocytes. Eotaxin-2 inhibits colony formation in myleloid progenitor cells.[1]

Reagents

Monoclonal Anti-Eotaxin-2 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-2 has the ability to neutralize the biological activity of recombinant human eotaxin-2.

To measure this activity, recombinant human eotaxin-2 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 human eotaxin-2 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 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 human 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-2 activity on a responsive cell line, when eotaxin-2 is present at a concentration just high enough to elicit a maximum response.

The Neutralization Dose$_{50}$ (ND_{50}) for this antibody is approximately 1 to 5 µg/ml in the presence of 100 ng/ml of recombinant human eotaxin-2, measuring the chemotaxis of rat myeloid Y3 cells transfected with the human CCR-3 gene.

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

For capture ELISAs, use 2 µg/ml of monoclonal anti-human eotaxin-2 (capture antibody). In the ELISA capture assay, plates are coated with 100 µl/well of the capture antibody (monoclonal anti-eotaxin-2) at 2 µl/ml in combination with 100 µl/well of the detection antibody (biotinylated, anti-human eotaxin-2 antibody) at 50 ng/ml.

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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