

Product Information

Anti-Lymphotactin

Developed in Goat
Affinity Isolated Antibody

Product Number **L 1165**

Product Description

Anti-Mouse Lymphotactin (Lptn) is developed in goat using a purified recombinant mouse lymphotactin expressed in *Escherichia coli* as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-lymphotactin antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Mouse Lymphotactin recognizes recombinant mouse lymphotactin by various immunochemical techniques including neutralization, immunoblotting, and ELISA. In ELISAs, this antibody shows less than 5% cross-reactivity with recombinant human GRO α , recombinant human GRO β , recombinant human GRO γ , and recombinant mouse MCP-5.

Mouse lymphotactin (Lptn)/XCL1 and its human homologue (also named human SCM-1 and ATAC), members of the γ or C subfamily of chemokines, are characterized by having only two cysteines. Mouse lymphotactin encodes a 114 amino acid precursor protein with a 21 amino acid predicted signal peptide. The gene for human and mouse lymphotactin has been mapped to chromosome 1. Human and mouse lymphotactin share approximately 60% amino acid sequence identity.

The expression of lymphotactin is restricted to activated mouse pro-T cells and to activated, class I MHC restricted T cells. Since lymphotactin is produced by lymphocytes and acts on lymphocytes, it is speculated that it is a messenger in T cell chemoattraction. Mouse lymphotactin is chemotactic for lymphocytes, NK cells, and mouse splenocytes.^{1,2} It has been shown that lymphotactin is a key regulator of lymphocyte motility and adhesion during acute allograft rejection.³ The specific receptor for lymphotactin is the orphan receptor XCR-1 (GPR5).

Reagent

Anti-Mouse Lymphotactin is supplied as 100 μ g of antibody lyophilized from a 0.2 μ m filtered solution of phosphate buffered saline (PBS).

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2 μ m filtered phosphate buffered saline to produce a 0.1 mg/ml stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at -20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Avoid repeated freezing and thawing. Do not store in "frost-free" freezer.

Procedure

Neutralization of Bioactivity

To measure the ability of anti-mouse lymphotactin to neutralize the chemoattractant activity of recombinant mouse lymphotactin for BaF/3 hXCR-1 transfected cells, recombinant mouse lymphotactin is incubated with various concentrations of the antibody for 15 minutes at room temperature in a 96 well plate. Following this pre-incubation, 75 μ l of the cytokine-antibody solution (containing recombinant mouse lymphotactin at a final concentration of 0.5 μ g/ml and antibody at concentrations from 0.1-200 μ g/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 2×10^6 cells/well is added to the top chamber. Following incubation for 3 hours at 37 °C in a 5% CO₂ humidified incubator, the chamber is carefully disassembled. The cells that migrate through to the lower chamber are transferred to a 96 well plate. Chemotaxis is measured by Alamar Blue staining of cells that have migrated through the filter.

Product Profile

Anti-Mouse Lymphotactin has the ability to neutralize the chemoattractant activity of recombinant mouse lymphotactin for BaF/3 human transfected cells (bone marrow derived cells transfected to express the lymphotactin receptor, XCR-1).

The Neutralization Dose₅₀ (ND₅₀) for anti-mouse lymphotactin is 5-15 µg/ml in the presence of 0.5 µg/ml of recombinant mouse lymphotactin using BaF/3 cells transfected with recombinant human XCR-1.

The ND₅₀ is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize recombinant mouse lymphotactin activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

For immunoblotting, a working antibody concentration of 1 to 2 µg/ml is recommended. The detection limit for recombinant mouse lymphotactin is approximately 5 ng/lane under non-reducing and reducing conditions.

For ELISAs, a working antibody concentration of 0.5 to 1.0 µg/ml is recommended. The detection limit for recombinant mouse lymphotactin is approximately 0.3 ng/well.

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

Endotoxin level is < 10 ng/mg antibody as determined by the LAL (Limulus amoebocyte lysate) method.

References

1. Kelner, G.S., et al., Lymphotactin: a cytokine that represents a new class of chemokine. *Science*, **266**, 1395-1399 (1994).
2. Hedrick, J.A., et al., Lymphotactin is produced by NK cells and attracts both NK cells and T cells *in vivo*. *J. Immunol.*, **158**, 1533-1540 (1997).
3. Wang, J.D., et al., Lymphotactin: a key regulator of lymphocyte trafficking during acute graft rejection. *Immunology*, **95**, 56-61 (1998).

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