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Product Information

Monoclonal Anti-RANTES, clone 53405.111
produced in rat, purified immunoglobulin

Catalog Number **R9400**

Product Description

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

Monoclonal Anti-RANTES recognizes recombinant mouse RANTES by various immunochemical techniques including immunoblotting, ELISA, and neutralization. From ELISA, this antibody shows ~50% cross-reactivity with recombinant human RANTES.

RANTES is a member of the β (C-C) chemokine subfamily, which also includes the human proteins MCP-1, MCP-2, MIP-1α, MIP1β, I-309, and their murine counterparts. Mouse RANTES cDNA encodes a 91 amino acid residue precursor polypeptide with a 23 amino acid residue hydrophobic signal peptide that is cleaved to produce the 68 amino acid residue mature protein, ~7.9 kDa. [1] Human and mouse RANTES share ~85 % amino acid identity.[1]

RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted) was first discovered by subtractive hybridization as a transcript expressed in T cells, but not B cells. Eosinophilic chemotactic substances released by thrombin-stimulated human platelets are identical to RANTES. In addition to T cells and platelets, RANTES is expressed by renal tubular epithelium [2], synovial fibroblasts, and selected tumor cells (rhabdomyosarcoma cells and MG63 osteosarcoma cells). The mouse RANTES gene maps to chromosome 11. [3]

The chemokine RANTES is a chemoattractant for monocytes [4], T lymphocytes [5], and NK (natural killer) cells. [6] RANTES selectively attracts T cells of the $CD4^+/CD45RO^+$ phenotype *in vitro*. [4] RANTES can also chemoattract and degranulate eosinophils [7], as well as chemoattract and induce histamine release from basophils.[8] RANTES has been shown to inhibit HIV-1 entry via human CCR-5.[9] RANTES and MIP-1α bind to chemokine receptors (CCR1, CCR4, and CCR5) and induce activation of STAT signaling pathways.[10] Because of its *in vitro* biological activities, RANTES may play an important role in mediating immune and inflammatory responses.

Reagent

Supplied as a lyophilized powder from a 0.2 μm filtered solution in phosphate buffered saline, pH 7.4, containing 5% trehalose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline (PBS), pH 7.4, 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 2-8 °C for at least one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Product Profile

Neutralization: Monoclonal Anti-RANTES is measured by its ability to neutralize the biological activity of recombinant mouse RANTES. This antibody will also neutralize the bioactivity of recombinant human RANTES when used at 100x higher IgG concentration.

To measure this activity, recombinant mouse RANTES 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 RANTES at a concentration of 0.05 μg/ml and antibody at concentrations from 0.001 to 10 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 (8 μm pore size) and 1 x 10^6 cells/well (2 day cultured human monocytes) is added to the top chamber. After incubation for 75 minutes at 37 °C in a 5 % CO_2 humidified incubator, the chamber is disassembled and the filter is fixed and stained. The optical density of the filter, which is proportional to the number of cells that migrate across the filter, is read on a microplate reader at 540 nm.

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

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

Immunocytochemistry: a working concentration of 8-25 μg/mL is recommended for detection in mouse splenocytes.

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

References

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