

## Reagents Provided

**Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CCL5/RANTES:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 21445

Isotype: mouse IgG<sub>1</sub>

## Reagents Not Provided

**Flow Cytometry Fixation Buffer** (Catalog # FC004) or other 4% paraformaldehyde fixation buffer

**Flow Cytometry Permeabilization/Wash Buffer I (1X)** (Catalog # FC005) or other saponin-containing saline buffer.

## Storage

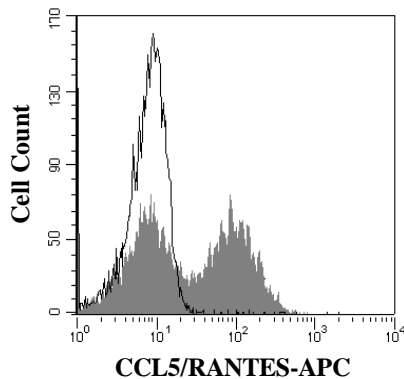
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells containing CCL5/RANTES within a population and qualitatively determine the density of intracellular CCL5/RANTES by flow cytometry.

## Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived, recombinant human CCL5 (rhCCL5). The IgG fraction from ascites fluids was purified by Protein A affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of CCL5/RANTES is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



NS0 transfected cells were stained with APC-conjugated anti-human CCL5/RANTES (Catalog # IC278A, filled histogram) or isotype control (Catalog # IC002A, open histogram).

## Background Information

CCL5, also known as RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted) is a member of the β (C-C) chemokine subfamily. It binds and activates the chemokine receptors CCR1, 3 and 5.

## Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see **Reagents Not Provided**).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. 5 x 10<sup>5</sup> cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 µL of conjugated antibody was added and cells were incubated for 30 minutes at room temperature **in the dark**.
6. Cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG, antibody. This procedure may need to be modified, depending on cell type and final utilization. Individual users may need to titrate to determine optimal reagent amount for their specific use.

**Warning:** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.