

## Reagents Provided

**Allophycocyanin (APC)-conjugated mouse monoclonal anti-human/rat CCR4:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 205410

**Isotype:** mouse IgG<sub>2b</sub>

## Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

## Storage

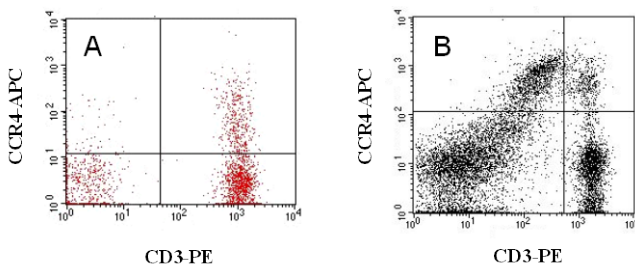
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing CCR4 within a population and qualitatively determine the density of CCR4 on cell surfaces 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 human CCR4 (Accession # P51679) transfectants. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of CCR4 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.



A) Human peripheral blood lymphocytes were stained with APC-conjugated anti-human/rat CCR4 (Catalog # FAB1567A) and PE-conjugated anti-human CD3 (Catalog # FAB100P).

B) Rat splenocytes were stained with APC-conjugated anti-human/rat CCR4 (Catalog # FAB1567A) and PE-conjugated anti-rat CD3. Quadrant markers were set based on isotype control staining (Catalog # IC003A).

## Background Information

CCR4 is a G-protein linked seven transmembrane spanning chemokine receptor that binds the chemokines CCL17/TARC and CCL22/MDC. Current evidence suggests that CCR4 expression is associated with Th2 type T cells and with platelets. CCR4 expression has also been reported in mature dendritic cells.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using hCCR4- transfected L1.2 cells, human peripheral blood cells, and rat splenocytes.

- Cells may be Fc-blocked with 1 µg of human or rat IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to up to 1 x 10<sup>6</sup> cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG<sub>2b</sub> antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine the 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.