

## Reagents Provided

**Carboxyfluorescein-conjugated goat polyclonal anti-mouse IL-17 RC:** Supplied as 75 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

**Isotype:** goat IgG

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

## Storage

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

## Intended Use

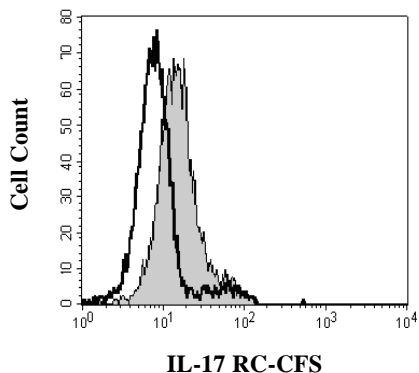
Designed to quantitatively determine the percentage of cells bearing IL-17 RC within a population and qualitatively determine the density of IL-17 RC on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the fluorescein-labeled polyclonal antibody, which binds to cells expressing IL-17 Rc. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing IL-17 RC are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IL-17 RC. Cell surface expression of IL-17 RC is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.

## Reagent Preparation

**Fluorescein-conjugated goat anti-mouse IL-17 RC:** Use as is; no preparation necessary.



Mouse RAW264.7 macrophages were stained with CFS-conjugated anti-mouse IL-17 RC (Catalog # FAB2270F, filled histogram) or isotype control (Catalog # IC108F, open histogram).

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FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of mouse IgG/10<sup>5</sup> cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of CFS-conjugated IL-17 RC reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted IL-17 RC reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with CFS-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

## Background Information

Interleukin 17 RC (also IL-17 RL) is a newly discovered member of the IL-17 receptor family.<sup>1,2</sup> There are currently five known family members, and all are type I transmembrane proteins. In addition, all show alternate splice forms, save for IL-17 R. In mouse, IL-17 RC is presently reported to be synthesized as a 698 amino acid (aa) precursor that contains a 20 aa signal sequence, a 443 aa extracellular region, a 20 aa transmembrane segment, and a 214 aa cytoplasmic domain.<sup>3</sup> There are multiple potential N-linked glycosylation sites in the extracellular region and potential phosphorylation sites in the cytoplasmic tail. Isoforms are presumed to exist for the mouse protein. R&D Systems recombinant mouse IL-17 RC product is an unusual 567 aa isoform.<sup>4</sup> Its precursor contains a 21 aa signal sequence, a 444 aa extracellular region, a 20 aa transmembrane segment and an 83 aa cytoplasmic tail.<sup>5</sup> Relative to the full length mouse IL-17 RC form, its extracellular region shows 99% aa identity, with an additional 24 aa insert. In the cytoplasmic region, it is highly divergent and shows virtually no aa identity.<sup>3,4</sup> The extracellular region of mouse IL-17 RC shows about 70% aa identity to the equivalent region in human IL-17 RC isoform # 3. The receptor has a general expression pattern, but apparently is not present in peripheral blood cells. In human prostate tumors, IL-17 RC is preferentially expressed in stromal elements.<sup>3</sup> While no ligand has yet been reported for IL-17 RC, soluble forms of the receptor will presumably act as an antagonist to ligand activity.<sup>3</sup>

## References:

1. Moseley, T.A. *et al.*, 2003, Cytokine Growth Factor Rev. **14**:155.
2. Kolls, J.K. and A. Linden, 2004, Immunity **21**:467.
3. Haudenschild, D. *et al.*, 2002, J. Biol. Chem. **277**:4309.
4. GenBank Accession # AAH04759.

**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.