

## Reagents Provided

**Phycoerythrin (PE)-conjugated goat polyclonal anti-mouse IL-4 R $\alpha$ :**  
Supplied as 25  $\mu$ g of antibody in 1 mL saline containing up to 0.5% BSA and 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

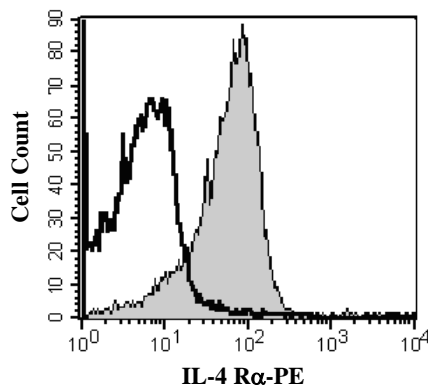
Designed to quantitatively determine the percentage of cells bearing IL-4 R $\alpha$  within a population and qualitatively determine the density of IL-4 R $\alpha$  on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled polyclonal antibody, which binds to cells expressing IL-4 R $\alpha$ . Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing IL-4 R $\alpha$  are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IL-4 R $\alpha$ . Cell surface expression of IL-4 R $\alpha$  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 565 - 605 nm.

## Reagent Preparation

**Phycoerythrin-conjugated goat anti-mouse IL-4 R $\alpha$ :** Use as is; no preparation necessary.



Mouse splenocytes were stained with PE-conjugated anti-mouse IL-4 R $\alpha$  (Catalog # FAB530P, filled histogram) or PE-conjugated isotype control (Catalog # IC108P, open histogram).

## 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  $\mu$ L 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  $\mu$ L 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  $\mu$ g 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  $\mu$ L of the Fc-blocked cells (up to 1 x 10<sup>6</sup> cells) or 50  $\mu$ L of packed whole blood to a 5 mL tube.
- 3) Add 10  $\mu$ L of PE-conjugated IL-4 R $\alpha$  reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted IL-4 R $\alpha$  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  $\mu$ L of PBS buffer for final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with PE-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

## Background Information

Interleukin 4 is a pleiotropic cytokine produced primarily by activated T cells, mast cells and basophils. The diverse biological effects of IL-4 on a variety of cell types are mediated by the binding of IL-4 to specific cell surface receptors. As is the case with many other cytokines, the functional high-affinity receptor for IL-4 is a complex consisting of a ligand binding subunit and a second subunit ( $\beta$  chain) that can modulate the ligand binding affinity of the receptor complex. It has been shown that in certain cell types, the gamma chain of the IL-2 receptor is a functional component ( $\beta$  chain) of the IL-4 receptor complex.

cDNA clones for the ligand binding chain (IL-4 R) of both the mouse and human high affinity IL-4 receptors have been isolated. The human or mouse IL-4 R is an approximately 140 kDa transmembrane protein containing an extracellular domain, a transmembrane domain, and a large cytoplasmic domain that is essential for IL-4 signal transduction. In addition to the cDNA clone encoding the full-length transmembrane protein, a second cDNA clone that arises from alternate splicing encodes a soluble secreted form of IL-4 R that has been isolated from mouse cells, but not yet from human sources. A naturally-occurring soluble form of the IL-4 R has also been identified in mouse biological fluids and in murine cell culture supernatants.

Native or recombinant murine soluble IL-4 R, as well as recombinant human soluble IL-4 R, can bind IL-4 with the same affinity as the membrane bound IL-4 R. Soluble IL-4 R is a competitive inhibitor of IL-4 and has been shown to effectively neutralize many IL-4-mediated responses both *in vivo* and *in vitro*.

**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.