Polyclonal
Anti-human IL-31 RA-Phycoerythrin

Catalog Number: FAB2769P
Lot Number: AAOY01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-human IL-31 RA: Supplied as 25 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

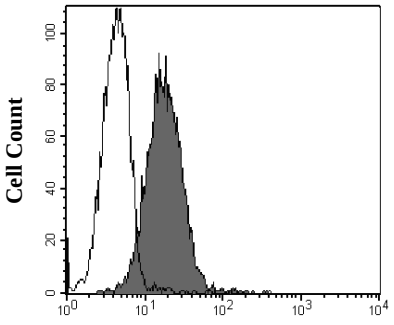
Designed to quantitatively determine the percentage of cells bearing IL-31 RA within a population and qualitatively determine the density of IL-31 RA on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled polyclonal antibody, which binds to cells expressing IL-31 RA. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing IL-31 RA are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IL-31 RA. Cell surface expression of IL-31 RA is determined by flow cytometry 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-human IL-31 RA: Use as is; no preparation necessary.



U937 cells were stained with PE-conjugated anti-human IL-31 RA (Catalog # FAB2769P, filled histogram) or 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) followed by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells should then be transferred to a 5 mL tube for staining with the polyclonal 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 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates 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 human IgG/10^5 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 (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated IL-31 RA reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted IL-31 RA 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 analysis by flow cytometry.
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.

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Background Information

The interleukin-31 receptor A subunit (IL-31 RA), also known as gp130-Like Monocyte Receptor (GLM-R or GPL), is a ~100 kDa type I transmembrane glycoprotein that is classified as being a type I cytokine receptor (1, 2). A heterodimeric complex of IL-31 RA and the oncostatin M receptor (OSM-R) functions as the signaling receptor for IL-31 (3). Both subunits are inducibly expressed throughout the myelomonocytic lineage and are upregulated by interferon-γ and bacterial lipopolysaccharides (1 - 3). IL-31 RA is also expressed on keratinocytes, dorsal root ganglia neurons, and variably on lung epithelial cells (3 - 6). The 732 amino acid (aa) IL-31 RA contains a 19 aa signal sequence, a 500 aa extracellular domain (ECD), a 21 aa transmembrane domain and a 192 aa cytoplasmic domain. The ECD shares 60%, 58%, 73% and 70% aa identity with mouse, rat, dog and cow IL-31 RA ECD, respectively. Human IL-31 receptors do not respond to mouse IL-31 (7). The ECD contains five fibronectin type III domains; the first two contain four conserved cysteine residues and a WSXWS motif common to type I cytokine receptors (2). Twelve alternately spliced human IL-31 RA isoforms are known and range in size from 356 - 745 amino acids. A long (745 aa) and a short (560 aa) transmembrane form are the predominant forms, and many cell lines express both forms (8). The long form, like the 732 aa form, signals by recruiting STAT3, 5 or 1, while the short form does not recruit STATs and inhibits IL-31 signaling. The ratio of these forms and their co-expression with OSM-R determine a cell's response to IL-31 (8). In both humans and transgenic mice, IL-31 from skin-homing Th2 cells may contribute to the pruritis (itching) associated with nonatopic dermatitis, especially in infected skin (3, 9, 10).

References

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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.