Polyclonal Anti-human IL-17 RD-Phycoerythrin

Catalog Number: FAB2275P
Lot Number: AACU02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-human IL-17 RD: Supplied as 25 μg 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

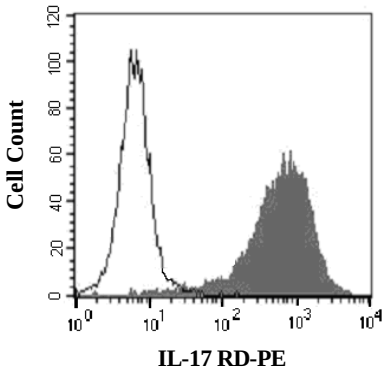
Designed to quantitatively determine the percentage of cells bearing IL-17 RD within a population and qualitatively determine the density of IL-17 RD on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled polyclonal antibody, which binds to cells expressing IL-17 RD. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing IL-17 RD are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IL-17 RD. Cell surface expression of IL-17 RD 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

Phycoerythrin-conjugated goat anti-human IL-17 RD: Use as is; no preparation necessary.



K562 cells were stained with PE-conjugated anti-human IL-17 RD (Catalog # FAB2275P, 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) 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 10^6 cells/mL and 25 μL of cells (1 x 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 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 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 (up to 1 x 10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated IL-17 RD reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted IL-17 RD 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 PE-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

Interleukin -17 receptor D (IL-17 RD), also known as SEF (similar expression to FGFs), is a type I transmembrane protein that is found in both the cytoplasm and plasma membrane (1 - 5). The gene for this protein belongs to a synexpression group originally identified in zebrafish and SEF is expressed along with FGF-3, -8, sprouty-2 (SPRY2) and SPRY4 (6, 7). By alternate splicing, two transcript variants, potentially encoding three protein isoforms exist. One is a full-length long form, one a shortened form that uses an alternate start site, and one an alternate splice form that removes the classic signal sequence (1 - 4). These isoforms have different expression patterns, subcellular localization, and function. The membrane-bound long form of human IL-17 RD is synthesized as a 739 amino acid (aa) precursor protein with a putative 27 aa signal peptide, a 272 aa extracellular domain, a 20 aa transmembrane segment and a 420 aa cytoplastic domain. The extracellular domain contains one Ig-like domain and a fibronectin type III motif. The cytoplasmic domain shares homology with the intracellular domains of IL-17 receptor family members and shows one TIR (Toll/IL-1 Receptor) domain and a putative TRAF6-binding motif (2). Natural IL-17 RD has been shown to form homomultimeric complexes (3). Unlike the alternate splice form of IL-17 RD that has a restricted pattern of expression, the full-length IL-17 RD isoform is expressed in most adult tissues and during embryonic development (3, 5). Functionally, IL-17 RD has been shown to be an inhibitor of FGF signaling. The molecule's extracellular domain does not seem to be involved. There is an interaction between the intracellular domains of FGF R1/FGF R2 and IL-17 RD that blocks ERK dissociation from MEK, thereby interfering with downstream ERK activation of nuclear Elk-1 (8). IL-17 RD has also been reported to interact with TAK1 and induce JNK activation and apoptosis (9). Ligands that interact with the extracellular domain of IL-17 RD have not been identified.

References

1. Furthauer, M. *et al*., 2002, Nat. Cell Biol. **4**:170.
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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.