

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human

IL-6 R: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 17506

Isotype: mouse IgG₁

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

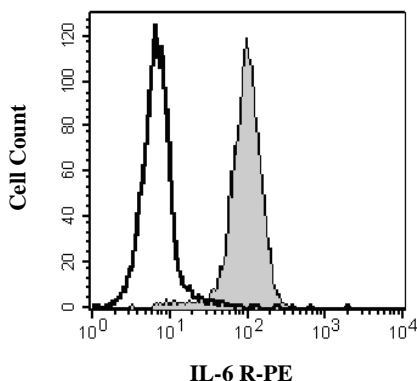
Designed to quantitatively determine the percentage of cells bearing IL-6 R within a population and qualitatively determine the density of IL-6 R on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing IL-6 R. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing IL-6 R are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IL-6 R. Cell surface expression of IL-6 R 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 mouse anti-human IL-6 R: Use as is; no preparation necessary.



U937 cells were stained with PE-conjugated anti-human IL-6 R

(Catalog # FAB227P, filled histogram) or isotype control

(Catalog # 1C002P, 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 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ 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 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated IL-6 R reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted IL-6 R reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysing 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 mouse IgG₁ antibody.

This procedure may need modification, depending upon final utilization.

Background Information

The multifunctional factor interleukin 6 (IL-6) exerts its activities through binding to a high-affinity receptor complex consisting of two membrane glycoproteins: an 80 kDa component receptor that binds IL-6 with low affinity (IL-6 R) and a signal-transducing component of 130 kDa (gp130) that does not bind IL-6 by itself, but is required for high-affinity binding of IL-6 by the complex. Both components of the receptor complex, IL-6 R and gp130 have been cloned, sequenced, and expressed (Yamasaki *et al.*, 1988, *Science* **241**:825; Baumann *et al.*, 1990, *J. Biol. Chem.* **265**:19853; Hibi *et al.*, 1990, *Cell* **63**:1149; Schooltink *et al.*, 1991, *Eur. J. Biochem.* **277**:659).

A soluble form of the IL-6 R has been found in the urine of healthy adult humans (Novick *et al.*, 1989, *J. Exp. Med.* **170**:1409). This soluble receptor apparently arises from proteolytic cleavage of membrane-bound IL-6 R. No naturally-occurring mRNA encoding a truncated form of the IL-6 R has been reported. Soluble forms of human and murine IL-6 Rs have been constructed, however, by insertion of termination codons into the regions of the IL-6 R cDNAs encoding the external portions of the receptors prior to the transmembrane domains. These soluble receptors have been expressed in COS-7 and CHO cells and have been shown to bind to IL-6 in solution and to augment the activity of IL-6 as a result of the binding of the IL-6/IL-6 R complex to membrane-bound gp130 (Yasukawa *et al.*, 1990, *J. Biochem.* **108**:673; Saito *et al.*, 1991, *J. Immunology* **147**:168).

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.