

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-human

TGF- β RIII: Supplied as 50 μ 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 TGF β RIII within a population and qualitatively determine the density of TGF- β RIII on cell surfaces by flow cytometry.

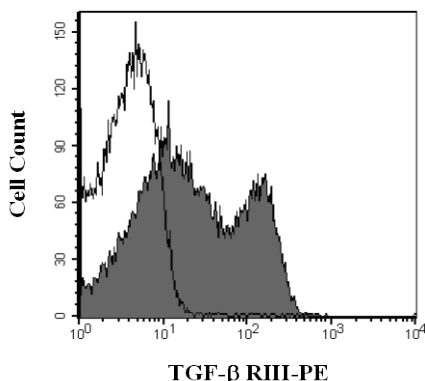
Principle of the Test

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

Use as is; no preparation necessary.



Human lymphocytes were stained with PE-conjugated anti-human TGF- β RIII (Catalog # FAB242P, filled histogram) or isotype control (Catalog # IC108P, open histogram).

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 μ 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 10⁶ cells/mL and 25 μ L 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 μ g 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 μ L of the Fc-blocked cells (up to 1 x 10⁶ cells) or 50 μ L of packed whole blood to a 5 mL tube.
- 3) Add 10 μ L of PE-conjugated TGF- β RIII reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted TGF β RIII 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 μ 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

Most cell types express three different sizes of TGF- β receptors. These are designated Type I (53 kDa), Type II (70 - 85 kDa), and Type III (250 - 350 kDa). The Type I receptor is a membrane-bound serine/threonine kinase that apparently requires the presence of the Type II receptor to bind TGF- β . The Type II receptor is also a membrane-bound serine/threonine kinase that binds TGF- β 1 and TGF- β 3 with high affinity and TGF- β 2 with a much lower affinity. The Type I and Type II receptors together form a heterodimeric signaling complex that is essential for the transduction of the anti-proliferative signals of TGF- β .

The Type III receptor is a transmembrane proteoglycan with a large extracellular domain and a 43 amino acid residue cytoplasmic domain. The cytoplasmic domain of the Type III receptor lacks an obvious signaling motif and the receptor may not be involved directly in signal transduction. The Type III receptor binds TGF- β 2 with the highest affinity. Other TGF- β isoforms also bind the Type III receptor, but with lower affinities. Cellular responsiveness to TGF- β 2 appears to be dependent on the presence of the Type III receptor which can interact with the signaling receptor complex. In addition to the transmembrane Type III receptor, a soluble form of the receptor is secreted by some cell types. The physiological role of this soluble receptor remains to be determined. The recombinant TGF- β soluble receptor Type III binds the TGF- β isoforms differentially in solution and exhibits TGF- β antagonistic activities *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.