Monoclonal Anti-human Thrombopoietin R-Allophycocyanin

Catalog Number: FAB1016A
Lot Number: AATJ02
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human Thrombopoietin R: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 167639

Isotype: mouse IgG_{2A}

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

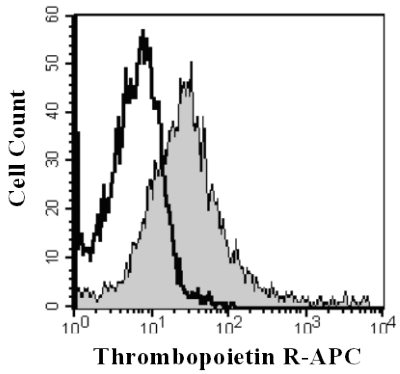
Designed to quantitatively determine the percentage of cells bearing Thrombopoietin R within a population and qualitatively determine the density of Thrombopoietin R on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing Thrombopoietin R. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing Thrombopoietin R are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Thrombopoietin R. Cell surface expression of Thrombopoietin R is determined by flow cytometry using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

Allophycocyanin-conjugated mouse anti-human Thrombopoietin R: Use as is; no preparation necessary.



TF-1 cells were stained with APC-conjugated anti-human Thrombopoietin R (Catalog # FAB1016A, filled histogram) or APC-conjugated isotype control (Catalog # IC003A, 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 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) 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 (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated Thrombopoietin R reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Thrombopoietin R 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 this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2A} 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

Thrombopoietin receptor (Tpo R), also known as myeloproliferative leukemia protein (c-mpl), is a 95 kDa type I transmembrane protein that is a member of the type I cytokine receptor family within the hematopoietin/cytokine receptor superfamily.[1-4] The 635 aa full-length human Tpo R contains a 25 amino acid (aa) signal sequence, a 466 aa extracellular domain with a ligand binding domain and two fibronectin type III domains, a transmembrane (TM) domain and a cytoplasmic domain. The extracellular domain of human Tpo R shares 78%, 76%, 81%, 82% and 80% aa identity with mouse, rat, cow, dog and horse Tpo R, respectively. Humans produce three distinct mRNA species; a P-form, a K-form, and a truncated form (Mpl-tr) lacking a TM domain.[3-7] The P-form encodes the full-length receptor. The Mpl-tr form, which is expressed in both human and mouse, is intracellular and targets the P-form for degradation.[5,6] The 579 aa K-form has an alternate cytoplasmic domain, but does not dimerize with, or inhibit, the P-form.[7] Thrombopoietin (Tpo) is a key regulator of megakaryocytopoiesis, thrombopoiesis and hematopoietic stem cell self-renewal, as reflected by expression of the Tpo R on megakaryocytes, platelets and hematopoietic progenitors.[2,8] Receptor dimerization occurs upon Tpo binding and initiates signaling through the Ras/MAP and JAK/STAT pathways.[1,2] Internalization and degradation of Tpo following Tpo R binding serves to down-regulate circulating Tpo.[9] Tpo R expressed at low levels on endothelial cells does not appear to contribute to regulation of Tpo.[10] Inactivating mutations of Tpo R cause thrombocytopenia, and absence of functional Tpo R is lethal in humans, but not mice. Other mutations, including an activating change in the TM domain, can cause thrombocytosis.[11,12]

References

1. Kaushansky, K. (2005) J. Clin. Invest. **115**:3339.
2. Deutsch, V.R. & A. Tomer (2006) Br. J. Haematol. **134**:453.
3. Vigon, I. *et al*. (1992) Proc. Natl. Acad. Sci. USA **89**:5640.
4. Mignotte, V. *et al*. (1994) Genomics **20**:5.
5. Li, J. *et al*. (2000) Cytokine **12**:835.
6. Coers, J. *et al*. (2004) J. Biol. Chem. **279**:36397.
7. Millot, G.A. *et al*. (2002) Exp. Hematol. **30**:166.
8. Tong, W. *et al*. (2007) Exp. Hematol. July 14 [Epub ahead of print].
9. Li, J. *et al*. (199) Br. J. Haematol. **106**:345.
10. Geddis, A.E. *et al.* (2006) Exp. Hematol. **34**:82.
11. Germeshausen, M. *et al*. (2006) Hum. Mutat. **27**:296.
12. Ding, J. *et al.* (2004) Blood **103**:4198.

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.