Monoclonal Anti-human Transferrin Receptor/CD71-Phycoerythrin

Catalog Number: FAB2474P
Lot Number: LQP02
100 Tests

Reagents Provided

Phycoerythrin-conjugated monoclonal anti-human transferrin receptor (TfR)/CD71: Supplied as 25 μg of antibody in 1 mL PBS containing 0.09% sodium azide.

Clone #: 29806

Isotype: mouse IgG_1

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

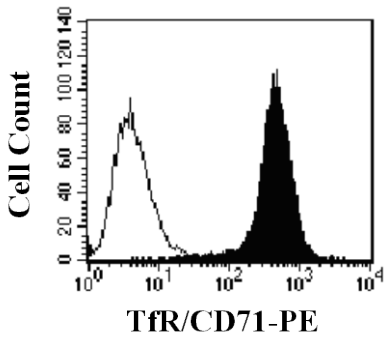
Designed to quantitatively determine the percentage of cells bearing TfR/CD71 within a population and qualitatively determine the density of TfR/CD71 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing TfR/CD71. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing TfR/CD71 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of TfR/CD71. Cell surface expression of TfR/CD71 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human TfR/CD71: Use as is; no preparation necessary.



U937 cells stained with anti-Transferrin Receptor/CD71-PE (R&D Systems, Catalog # FAB2474P, filled histogram) or isotype control (R&D Systems, Catalog # IC002P, 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 4×10^6 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 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 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-TfR reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-TfR 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 mouse IgG_1 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

Transferrin receptor (TfR), CD71 or transferrin receptor 1, is the major mediator of iron uptake by cells (1, 2). The TfR is a transmembrane, disulfide-linked dimer of two identical subunits (3 - 7) that binds and internalizes diferric transferrin, thereby delivering iron to the cell cytosol. When a cell needs iron, TfR expression is increased to facilitate iron uptake (8 - 10). Since the major use of iron is for hemoglobin synthesis, about 80% of total TfR/CD71 is on erythroid progenitor cells (1, 2). Transferrin receptors are also highly expressed on placental tissue, and rapidly dividing cells, both normal and malignant (11 - 13). Its wide cellular expression pattern reflects the need for iron in cellular proliferation as iron is essential for sustaining ribonucleotide reductase activity (14). A soluble form of the receptor sTfR arises from the proteolysis of TfR at a specific site in the extracellular domain, leading to monomer units (15, 16). Measurement of sTfR is valuable as an indication of iron deficiency in individuals with chronic disease (inflammatory diseases, infections, malignancies), many of which are anemic.

References

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Warming: 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.