Monoclonal Anti-human Leptin R-Fluorescein

Catalog Number: FAB867F
Lot Number: BAAJ03
100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human Leptin R: Supplied as 50 μg of antibody in 1 mL PBS containing 0.09% sodium azide.

Clone #: 52263

Isotype: mouse IgG_{2B}

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

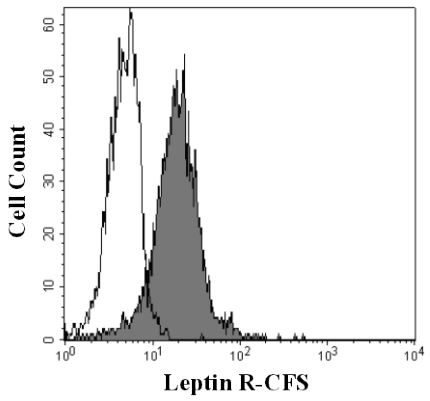
Designed to quantitatively determine the percentage of cells bearing Leptin R within a population and qualitatively determine the density of Leptin R on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing Leptin R. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing Leptin R are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Leptin R. Cell surface expression of Leptin 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 515 - 545 nm.

Reagent Preparation

Fluorescein-conjugated mouse anti-human Leptin R: Use as is; no preparation necessary.



Human monocytes were stained with CFS-conjugated anti-human Leptin R (Catalog # FAB867F, filled histogram) or isotype control (Catalog # IC0041F, 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 CFS-conjugated Leptin R reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Leptin 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 final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with CFS-labeled mouse IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

R&D Systems Inc.
1-800-343-7475

Background Information

Leptin receptor, also known as OB-R, is a type I cytokine receptor family protein that functions as a receptor for Leptin, an adipocyte-specific hormone that regulates adipocyte tissue mass through hypothalmic effects on satiety and energy expenditure.

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.