Monoclonal Anti-mouse P-Cadherin-Phycoerythrin

Catalog Number: FAB761P
Lot Number: LXG02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse P-cadherin: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 106020

Isotype: rat 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

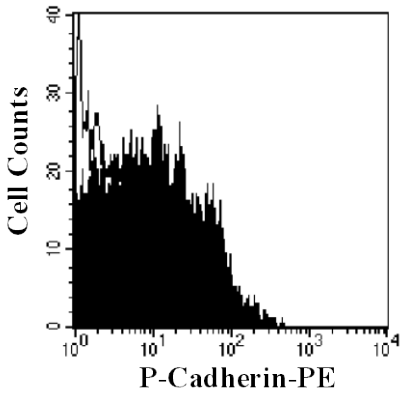
Designed to quantitatively determine the percentage of cells bearing P-cadherin within a population and qualitatively determine the density of P-cadherin on cell surfaces by flow cytometry.

Principle of the Test

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

Reagent Preparation

Phycoerythrin-conjugated rat anti-mouse P-cadherin: Use as is; no preparation necessary.



Mouse XB-2 cells were stained with PE-conjugated anti-mouse P-Cadherin (Catalog # FAB761P, filled histogram) or PE-conjugated isotype control (Catalog # IC006P, open histogram).

Sample Preparation

Tissues: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. 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. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells 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) are 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse IgG/10^5 cells for 15 minutes at room temperature. 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 PE-conjugated anti-P-cadherin reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove any unreacted anti-P-cadherin reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
6) 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 rat IgG_{2A} antibody.

This procedure may need to be modified, depending upon final utilization.

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Background Information

Placental (P) - Cadherin is a member of the Cadherin family of cell adhesion molecules. Cadherins are calcium-dependent transmembrane proteins that bind to one another in a homophilic manner. P-Cadherin has been designated Cadherin-3 (CDH3). Cadherins play a role in development, specifically in tissue formation.

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.