Monoclonal Anti-human Cadherin-8-Phycoerythrin

Catalog Number: FAB188P
Lot Number: LYY01
100 Tests

Reagents Provided

Phycoerythrin-conjugated mouse monoclonal anti-human Cadherin-8: Supplied as 50 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 147210

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

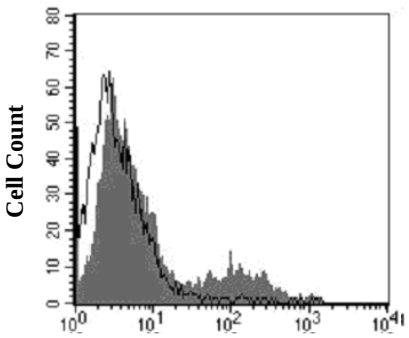
Designed to quantitatively determine the percentage of cells bearing Cadherin-8 within a population and qualitatively determine the density of Cadherin-8 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Cadherin-8. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Cadherin-8 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Cadherin-8. Cell surface expression of Cadherin-8 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 mouse anti-human Cadherin-8: Use as is; no preparation necessary.



Caki-2 cells with Ca^{++} and Mg^{++} were stained with anti-human Cadherin-8 (R&D Systems, Catalog # FAB188P, filled histogram) or isotype control (R&D Systems, 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) 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 (1 x 10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of PE-conjugated Cadherin-8 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Cadherin-8 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_{2A} antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

Cadherin-8 is a member of the type II, also called atypical, subfamily of classic Cadherin cell adhesion molecules. Cadherins are transmembrane calcium-dependent cell adhesion proteins. On their cytoplasmic side, they associate with the three catenins α, β and γ (plakoglobin). This association links the Cadherin protein to the cytoskeleton. Type I Cadherins consist of a large extracellular domain with an N-terminal propeptide sequence that is proteolytically cleaved intracellularly, five Cadherin repeats, four calcium-binding pockets between the Cadherin repeats and single-pass transmembrane domain, and a short carboxy-terminal cytoplasmic domain responsible for interacting with the catenins. Within the N-terminal Cadherin domain, a conserved HAV motif involved in homophilic interaction is present. In contrast, the type II Cadherins do not contain the HAV motif in the N-terminal Cadherin domain and display weak or no cell adhesive properties. There also appears to be more diversity in the cytoplasmic domains of the type II Cadherins as compared to the type I Cadherins. Cadherin-8 is most highly expressed in neuronal tissues. The rat Cadherin-8 protein has been suggested to play a role in long-term potentiation. Human Cadherin-8 is a 799 amino acid (aa) residue protein with a putative 29 aa signal sequence, and a 32 aa propeptide, a 560 aa mature extracellular domain, a 21 aa transmembrane domain and a 157 aa cytoplasmic domain. The human, mouse and rat proteins share approximately 98% homology. (Tanihara, H. *et al*., 1994, Cell Adhes. Comm. **2**:15 - 26; Suzuki, S. *et al*., 1991, Cell Regul. **2**:261 - 270; Nollet, F. *et al*., 2000, J. Mol. Biol. **299**:551 - 572; Yamagata, K. *et al*., 1999, J. Biol. Chem. **274**:19473 - 19479; Kido, M. *et al*., 1998, Genomics **48**:186 - 194).

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.