Polyclonal
Anti-human Cadherin-11-Phycoerythrin

Catalog Number: FAB1790P
Lot Number: AAOQ02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-human Cadherin-11: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Antibody type: goat IgG

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

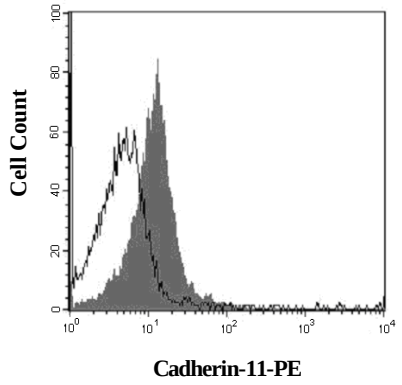
Designed to quantitatively determine the percentage of cells bearing Cadherin-11 within a population and qualitatively determine the density of Cadherin-11 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled polyclonal antibody, which binds to cells expressing Cadherin-11. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Cadherin-11 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Cadherin-11. Cell surface expression of Cadherin-11 is determined by flow cytometry 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 goat anti-human Cadherin-11: Use as is; no preparation necessary.



PC-3 cells were stained with PE-conjugated anti-human Cadherin-11 (Catalog # FAB1790P, filled histogram) or isotype control (Catalog # IC108P, open histogram).

フナコシ株式会社
試薬に関して: Tel. 03-5684-1620 / Fax 03-5684-1775
e - mail : reagent@funakoshi.co.jp

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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 polyclonal 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 (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated Cadherin-11 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Cadherin-11 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 PE-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

Background Information

The Cadherin superfamlily comprises a large number of membrane glycoproteins with one or more Cadherin repeats, which are involved in Ca^{2+} dependent cell-cell adhesion. The family can be subdivided into several major subgroups, including the type I and type II classical Cadherins, desmosomal Cadherins, protoCadherins, seven transmembrane (Flamingo) Cadherins, FAT-family Cadherins, T-Cadherin and other unclassified Cadherins.[1] Cadherin-11, also known as OB-Cadherin, is a type II classical Cadherin. Classical Cadherins are type I transmembrane proteins with an N-terminal extracellular domain containing five tandem Cadherin repeats and a C-terminal cytoplasmic domain with a characteristic sequence for binding to catenins. Type I Cadherins (E-, N-, P-, R-, M-, and EP-Cadherin) differ from type II Cadherins (Cadherin-5 to -12, -18 to -20, and -22) by the presence of the HAV tripeptide motif in the most N-terminal Cadherin repeat.[2] Classic Cadherins mediate cell-cell adhesion preferentially via homotypic interactions and form adherens junctions that have β-catenin and p120 at the cytoplasmic side of the junction.[3, 4] Homotypic Cadherin interactions also transduce outside-in and inside-out cell signals. Cadherin signaling induces various cellular processes including cell motility, actin cytoskeleton reorganization, proliferation, and differentiation.[3, 4] Cadherin-11 is expressed in a variety of normal tissues of mesodermal origin including areas of the kidney and brain, in normal osteoblasts, and in tumors of the stomach, kidney, colon, breast, and bone (osteosarcoma).[5, 6] It is also differentially expressed in the embryonic brain and may be important in regulating neural development. Human Cadherin-11 exhibits a unique mRNA splice site allowing for two forms of the protein to be expressed, a full-length 796 amino acid (aa) protein and a COOH terminus-truncated variant of 693 aa. The truncated variant has a unique cytoplasmic region due to a frameshift event.[3] The full-length human and mouse Cadherin-11 share 97% homology at the aa sequence level.

References

1. Angst, B.D. *et al*. (2001) J. Cell Sci. **113**:629.
2. Gessner, R. & R. Tauber (2000) Ann. N.Y. Acad. Sci. **915**:136.
3. Feltes, C.M. *et al*. (2002) Cancer Research **62**:6688.
4. Wheelock, J.J. & K.R. Johnson (2003) Annu. Rev. Cell Dev. Biol. **19**:207.
5. Hoffmann, I & R. Balling (1995) Dev. Biol. **169**:337.
6. Pishvaian, M.J. *et al*. (1999) Cancer Research **59**:947.

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.