Monoclonal Anti-human CD151-Phycoerythrin

Catalog Number: FAB1884P
Lot Number: LWM02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CD151: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 210127

Ig class: mouse IgG_{2B}

Additional Reagents Required

- 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

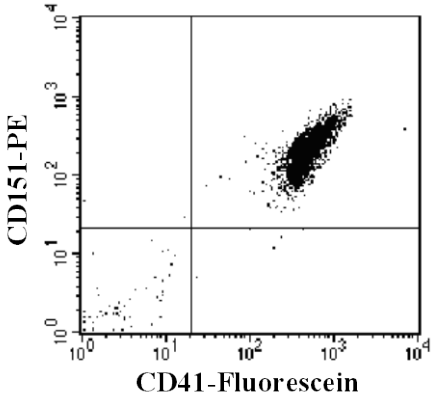
Designed to determine the percentage of cells expressing cell surface CD151 and the density of this protein on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing CD151. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing CD151 are fluorescently stained, with the intensity of staining directly proportional to the density of the CD151 protein. Cell surface expression of CD151 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human CD151: Use as is; no preparation is necessary.



Human peripheral blood platelets stained with Fluorescein-conjugated anti-human CD41 and PE-conjugated anti-human CD151 (Catalog # FAB1884P).

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. 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) 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 for 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 a 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 human 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 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-CD151 reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CD151 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 Whole Blood Lysing Kit, Catalog # WL1000).
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 mouse IgG_{2B} antibody.

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

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Background Information

CD151 is a 32 kDa member of the tetraspan superfamily of proteins (1, 2). Originally known as PETA-3 (platelet-endothelial tetraspan antigen-3), CD151 is primarily expressed on endothelium, platelets, and megakaryocytes (1, 2). The expression of CD151 on normal monocytes is reportedly very weak, but is highly expressed on a variety of myeloid leukemia cell lines (1). Other cells reported to express CD151 include epithelial cells and skeletal, smooth and cardiac muscle cells (3). Members of the tetraspan family, including CD151, homodimerize and form complexes with each other (4, 5). In addition, CD151 often is associated with integrin molecules (5-9). Specifically, interactions with $\alpha 3\beta 1$ (6, 7) as well as $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$, $\alpha IIb\beta 3$ (5) and $\alpha 6\beta 4$ (8, 9) have been demonstrated.

Functionally, the interaction of CD151 with such a variety of integrin molecules lends support to hypotheses that CD151 plays a role in intracellular cytoskeleton signalling and/or regulation of cell motility (7, 8). Potential inhibition of tumor cell motility may be possible in come cancers by modulating the expression of CD151 (9). Moreover, increased expression of CD151 has been correlated with poor prognosis in colon cancer (10).

References

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Warning: This reagent 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.