Monoclonal Anti-human D6-Phycoerythrin

Catalog Number: FAB1364P
Lot Number: LCM02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated rat monoclonal anti-human D6: Supplied as 50 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 196124

Ig Class: rat IgG_{2A}

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 quantitatively determine the percentage of cells bearing D6 within a population and qualitatively determine the density of D6 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody that binds to the cells expressing human D6. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing D6 are fluorescently stained, with the intensity of staining directly proportional to the density of D6. Cell surface expression of D6 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated rat anti-human D6: Use as is; no preparation is necessary.

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 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 or 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-human D6 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-D6 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 Human Erythrocyte 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 rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

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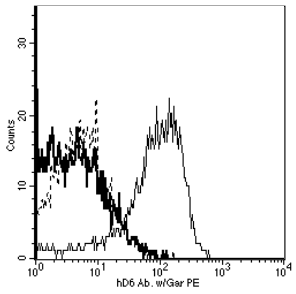
Background Information

The human D6 chemokine receptor is a member of the rhodopsin superfamily of G-linked receptors that span the cell membrane with seven hydrophobic transmembrane domains. It has greatest sequence homology (around 40%) to members of the β-chemokine receptor family (1). The D6 receptor has promiscuous binding characteristics since it appears to be able to bind most β-chemokines. However, it is unable to signal, likely as a result of amino acid changes present in key conserved domains of the receptor sequence (1, 2). Expression of the receptor has been reported on endothelial cell lining afferent lymphatics, placenta and a variety of primitive erythromyeloid cell lines (1 - 3). Of interest is the finding that over 70% of vascular tumors express D6 receptors (3). The most compelling evidence of the D6 receptor's biological function is found in a recent report showing that there is active internalization and degradation of chemokines (2). This finding would suggest that through its action as a non-activating decoy receptor, D6 may be capable of modulating the action of inflammatory chemokines.

References

1. Nibbs, R.J.B. *et al*. (1997) J. Biol. Chem. **273**:32078.
2. Fra, A.M. *et al*. (2003) J. Immunol. **170**:2279.
3. Nibbs, R.J.B. *et al*. (2001) Amer. J. Pathol. **158**:867.

Figure 1



The specificity of anti-human D6 monoclonal antibody (clone # 196124) was demonstrated by its ability to react with NS0 cells transfected with the human D6 receptor (*solid thin line*) and for its ability not to react with wild type (non-transfected) NS0 cells (*solid bold line*). Isotype control staining of the human D6 transfected cells is shown by the dotted line.

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.