

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-human/mouse Frizzled-3: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 169310

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 Frizzled-3 within a population and qualitatively determine the density of Frizzled-3 on cell surfaces by flow cytometry.

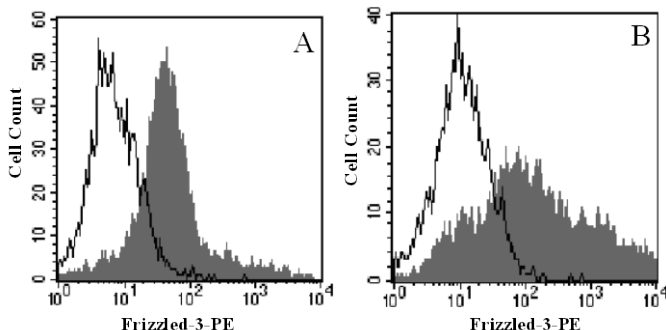
Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Frizzled-3. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Frizzled-3 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Frizzled-3. Cell surface expression of Frizzled-3 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 mouse anti-human Frizzled-3:

Use as is; no preparation necessary.



A) HEK293 cells and B) D3 cells treated with retinoic acid were stained with PE-conjugated anti-human/mouse Frizzled-3 (Catalog # FAB1001P, filled histogram) or PE-conjugated isotype control (Catalog # IC006P, 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) 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 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) 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 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ 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 (up to 1 x 10⁶ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated Frizzled-3 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted Frizzled-3 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 rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

The Wnt genes encode a large family of glycoproteins that are essential in development and tissue maintenance.¹ Members of the Frizzled family of proteins serve as receptors for the Wnt signaling pathway.² Frizzled-3 is an integral membrane protein that belongs to the Frizzled family of G-protein coupled receptors. Ten mouse and human Frizzled genes have been identified to date. The predicted structure of Frizzled proteins is similar among all family members, containing a divergent N-terminal signal peptide, a highly conserved extracellular cysteine-rich domain (CRD), a variable-length linker region, a seven-pass transmembrane region, and a variable-length C-terminal cytoplasmic domain. The CRD contains 642 amino acid residues and shares 95% identity with the CRD of the human orthologue. Several Frizzled-dependent signaling pathways exist.² Their activation depends on the Wnt ligand and the cell context. The low density lipoprotein receptor-related proteins (LRP) are co-receptors for the Wnt ligands. LRP5/6 serve as co-receptors in the Wnt/Frizzled canonical pathway that alters gene expression via the stabilization of β -catenin.^{4,5}

References

1. Wodarz, A., and R. Nusse (1998) *Annu. Rev. Cell Dev. Biol.* 14:59.
2. Hsieh, J-C. (2004) *Front. Biosci.* 9:1333.
3. Xu, L. *et al.* (2001) *Matrix Biol.* 2:147.
4. Tamai, K. *et al.* (2000) *Nature* 407:530.
5. Pinson, K. *et al.* (2000) *Nature* 407:535.

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.