

## Reagents Provided

**Phycoerythrin (PE)-conjugated rat monoclonal anti-human/mouse Frizzled-4:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 145901

**Isotype:** rat IgG<sub>2A</sub>

## 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-4 within a population and qualitatively determine the density of Frizzled-4 on cell surfaces by flow cytometry.

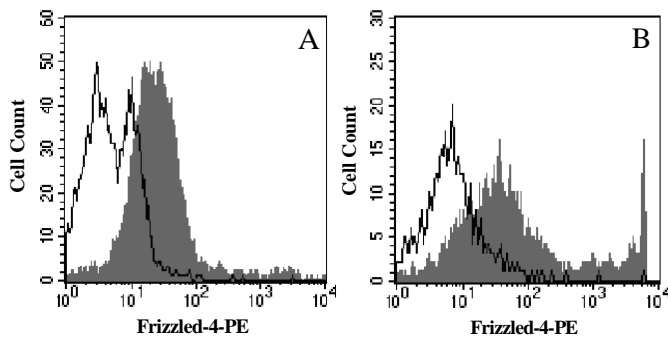
## Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Frizzled-4. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Frizzled-4 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Frizzled-4. Cell surface expression of Frizzled-4 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-4:

Use as is; no preparation necessary.



A) HEK293 cells and B) RA treated D3 cells were stained with PE-conjugated anti-human/mouse Frizzled-4 (Catalog # FAB194P, filled histogram) or isotype control (Catalog # IC006P, open histogram).

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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 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 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) 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<sup>5</sup> 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<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated Frizzled-4 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted Frizzled-4 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 analysis, cells in a separate tube should be treated with PE-labeled rat IgG<sub>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

## Background Information

Wnt signaling is involved in a variety of developmental processes including cell fate determination, cell polarity, tissue patterning, and control of cell proliferation. Members of the Frizzled family of proteins serve as receptors for the Wnt signaling pathway. The founding member of this family was identified in *Drosophila* based on its role in tissue polarity in the adult cuticle, and named for the disorganized appearance of bristle hairs on the mutant. Mouse Frizzled-4 (mFrz-4) was originally cloned in a PCR screen to identify additional Frizzled family members in vertebrates. There are nine murine Frizzled genes and 10 human Frizzled genes 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, a variable-length linker region, a seven-pass transmembrane region, and a variable-length C-terminal tail. The most conserved regions of the Frizzled proteins are the extracellular cysteine-rich domain (CRD) which spans approximately 120 amino acid (aa) residues and contains 10 invariant cysteines, and the seven transmembrane domains. Mouse Frizzled-4 is 537 aa long and shows 97% and 93% aa identity to human and *Xenopus* Frizzled-4, respectively.

Two distinct Wnt signal transduction pathways have been characterized. One is the canonical Wnt/ $\beta$ -catenin pathway that is involved in diverse biological mechanisms such as dorsal/ventral development in *Xenopus* embryos and mammalian tumor formation. Frizzled-4 is implicated in this pathway based on its ability to induce  $\beta$ -catenin target genes in the presence of ligand. However, Frizzled-4 is also implicated in the Wnt/ $\text{Ca}^{2+}$  flux pathway, another mechanism for Wnt signal transduction that does not rely on  $\beta$ -catenin activity, but on intracellular calcium fluxes. Since the CRD of mFrz-4, like mFrz-8, binds cell-surface *Xenopus* Wnt-8 (Xwnt-8) protein, this product is tested to antagonize Wnt signaling in the same way as R&D Systems mFrz-8/Fc: for inhibition of secondary axes induced by Xwnt-8 mRNA.<sup>1-4</sup>

## References

1. Wan, Y. *et al.* (1996) J. Biol. Chem. **271**:4468.
2. Hsieh, J-C. *et al.* (1999) Proc. Natl. Acad. Sci. USA **96**:3546.
3. Sheldahl, L. *et al.* (1999) Curr Biol. **9**:695.
4. Kirikoshi, H. *et al.* (1999) Biochem. Biophys. Res. Commun. **264**:955.

**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.