

Reagents Provided

PerCP-conjugated mouse monoclonal anti-human PDGF R β : Supplied as 25 μ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: PR7212

Isotype: mouse IgG₁

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

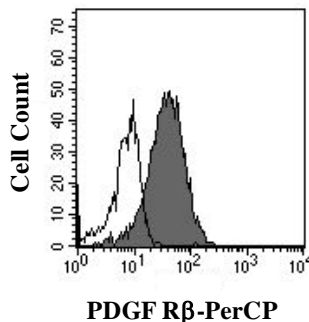
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing PDGF R β within a population and qualitatively determine the density of PDGF R β on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with human skin fibroblast membrane extracts. The IgG fraction of the ascites was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Cell surface expression of PDGF R β is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



Human MG-63 cells were stained with PerCP-conjugated anti-human PDGF R β (Catalog # FAB1263C; filled histogram) or PerCP-conjugated isotype control (Catalog # IC002C).

Background Information

PDGF is a major serum mitogen that can exist as a homodimeric or heterodimeric protein consisting of disulfide-linked PDGF-A and PDGF-B chains. The PDGF-AA, PDGF-BB, and PDGF-AB isoforms have been shown to bind to two distinct cell surface PDGF receptors with different affinities. Whereas PDGF R α binds all three PDGF isoforms with high affinity, PDGF R β binds only PDGF-BB with high affinity. Both PDGF R α and PDGF R β are members of the class III subfamily of receptor tyrosine kinases (RTK) that also includes the receptors for M-CSF, SCF, and Flt-3 ligand. All class III RTKs are characterized by the presence of five immunoglobulin-like domains in their extracellular region and a split kinase domain in their intracellular region. PDGF binding induces receptor homo- and hetero-dimerization and signal transduction. The expression of the α and β receptors is independently regulated in various cell types. Recombinant soluble PDGF R β binds PDGF with high affinity and is potent PDGF antagonist.⁴

References

- Hart, *et al.* (1987) J. Biol. Chem. **262**:10780.
- Gronwald, *et al.* (1988) Proc. Natl. Acad. Sci. USA **85**:3435.
- Seifert, *et al.* (1989) J. Biol. Chem. **264**:8771.
- Heldin, C.H. & L. Claesson-Welsh (1994) in *Guidebook to Cytokines and Their Receptors*, Nicola, N.A. ed. Oxford University Press, New York, p. 202.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using MG-63 cells.

- Cells may be Fc-blocked with 1 μ g of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 μ L of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG₁ antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

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.