Technical Data Sheet

Alexa Fluor® 488 Mouse anti-PDGFRβ (CD140b) (pY857)

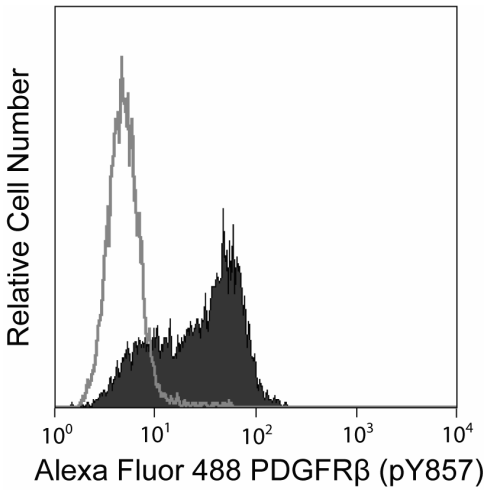
Product Information

Material Number:	**558427**
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	J24-618
Immunogen:	Phosphorylated Human PDGFRβ Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Platelet-derived growth factor (PDGF) is a potent mitogen for cells of mesenchymal origin and exerts its effects by binding to the PDGF receptor (PDGFR), a transmembrane protein tyrosine kinase. PDGFR is composed of PDGFRα (CD140a) and/or PDGFRβ (CD140b) polypeptides. Both PDGF and PDGFR consist of subunits that form homo- or heterodimers with varying specificities: PDGF-AA binds only to αα PDGFR, PDGF-AB binds to both αα and αβ PDGFR, and PDGF-BB binds to all three PDGFRs. Ligand binding induces dimerization and activation of the receptor. Upon activation, CD140b is phosphorylated at multiple tyrosine sites and, in turn, an intracellular phosphorylation cascade is initiated. PDGFR localizes primarily to membrane invaginations termed caveolae, compartments that are enriched in several of its downstream effectors, including phosphatidylinositol 3'-kinase, Src, and phospholipase C-γ.

The J24-618 monoclonal antibody recognizes the phosphorylated tyrosine 857 (pY857) in the tyrosine kinase domain 2 of CD140b, which is required for maximal receptor kinase activity. The orthologous phosphorylation site in mouse PDGFRβ is Y856.



Analysis of PDGFRβ (CD140b) (pY857) in mouse embryonic fibroblasts. *Serum-starved NIH/3T3 cells were either stimulated with PDGF-BB (Cat. No. 354051, shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Phosflow™ Fix Buffer I, Cat. No. 557870) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-PDGFRβ (CD140b) (pY857). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

Baxter RM, Secrist JP, Vaillancourt RR, Kazlauskas A. Full activation of the platelet-derived growth factor β-receptor kinase involves multiple events. *J Biol Chem.* 1998; 273(27):17050-17055. (Biology)

Chiarugi P, Cirri P, Taddei ML, Giannoni E, et al. Insight into the role of low molecular weight phosphotyrosin phosphatase (LMW-PTP) on platelet-derived growth factor receptor (PDGF-r) signaling. *J Biol Chem.* 2002; 277(40):37331-37338. (Biology)

Claesson-Welsh L. Platelet-derived growth factor receptor signals. *J Biol Chem.* 1994; 269(51):32023-32026. (Biology)

Liu J, Oh P, Horner T, Rogers RA, Schnitzer JE. Organized endothelial cell surface signal transduction in caveolae distinct from glycosylphosphatidylinositol-anchored protein microdomains. *J Biol Chem.* 1997; 272(11):7211-7222. (Biology)

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