# Fluorokine®
# Biotinylated Human PDGF-BB

**Catalog Number:** NFPDBB
**100 Tests**

## Reagents Provided

**Biotinylated rhPDGF-BB (100 reactions):** Liquid protein.

**2 mL Avidin-Fluorescein:** Avidin conjugated with fluorescein (10 μg/mL) at an f:p ratio of 5:1.

**600 μL Negative Control Reagent (60 reactions):** A protein (soybean trypsin inhibitor) biotinylated to the same degree as the cytokine (5 μg/mL).

**Blocking Antibody (15 reactions):** Polyclonal goat IgG anti-human PDGF-BB antibody.

**60 mL 10X Cell Wash Buffer:** A buffered saline-protein solution specifically designed to minimize background staining and stabilize specific binding.

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- human or mouse IgG

## Storage

**All Reagents:** 2 8° C

## Intended Use

Designed to quantitatively determine the percentage of cells expressing PDGF-BB receptors within a population, and to estimate the receptor density for PDGF-BB on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the biotinylated cytokine that in turn binds to the cells via specific cell surface receptors. The cells are then directly incubated with avidin-fluorescein, which attaches to the receptor-bound biotinylated cytokine. Unbound biotinylated cytokine participates in an amplification reaction with the bound cytokine that results in an enhanced signal without compromising specificity. Cells expressing the specific cytokine receptors are fluorescently stained, with the intensity of staining proportional to the density of the receptors. Relative receptor density is then determined by flow cytometry using 488 nm wavelength laser excitation.

## Reagent Preparation

**Biotinylated rhPDGF-BB:** Aliquot in sterile plastic vials. Store at -20 °C to -80 °C for up to 2 years. **Avoid repeated freeze-thaw cycles.**

**Blocking Antibody**: If lyophilized, reconstitute by adding 300 μL of sterile distilled water.

**1X Cell Wash Buffer:** Add 1 mL of 10X concentrate to 9 mL of sterile distilled water. Reagent should be maintained on ice during use and stored at 2° - 8° C for no more than 2 months.

## Sample Preparation

**Peripheral Blood Cells**: Whole blood collected in heparinized tubes should be processed by standard Ficoll-Hypaque gradient separation techniques to isolate mononuclear cells. Ficoll and contaminating serum components should be removed by washing the cells twice with PBS and then resuspending the cells in PBS or 1X Cell Wash Buffer. Cells should be resuspended to a final concentration of $4 \times 10^6$ cells/mL.

**Cultured Cells**: Cells from continuous or activated cultures should be centrifuged at 500 x g for 5 minutes and then washed twice with PBS to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in PBS to a final concentration of $4 \times 10^6$ cells/mL.

**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

Add 10 μL of biotinylated cytokine reagent to 25 μL of the washed cell suspension in a 12 x 75 mm tube for a total reaction volume of 35 μL. As a negative staining control, an identical sample of cells should be stained with 10 μL of biotinylated negative control reagent.

1. Incubate the cells for 30 - 60 minutes at 2° - 8° C.
2. Add 10 μL of avidin-FITC reagent to each tube. **(DO NOT WASH CELLS PRIOR TO ADDING AVIDIN-FITC)**.
3. Incubate the reaction mixture for an additional 30 minutes at 2° - 8° C **in the dark**.
4. Wash the cells twice with 2 mL of 1X Cell Wash Buffer to remove unreacted avidin-fluorescein and resuspend the cells in approximately 0.2 mL of 1X Cell Wash Buffer for final flow cytometric analysis.

## Specificity Testing

1. (Optional) Cells that are to be stained could be pretreated with purified mouse or human IgG (10 μL of 1 mg/mL/$10^6$ cells) for 15 minutes at room temperature in order to block Fc-mediated interactions. (*Cells should not be washed of excess IgG for this assay*).
2. **In a separate tube**, 20 μL of anti-human PDGF-BB blocking antibody is mixed with 10 μL of PDGF-BB-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-PDGF-BB blocking antibody and Fluorokine mixture, add $1 \times 10^5$ Fc-blocked cells in a volume of 25 μL.
4. The reaction is then allowed to proceed as described in steps 1 - 4 above.



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## Background Information

The Platelet-Derived Growth Factor (PDGF) family consists of proteins derived from four genes (PDGF-A, -B, -C and -D) that form four disulfide-linked homodimers (PDGF-AA, -BB, -CC, -DD) and one heterodimer (PDGF-AB).[1 - 3] Most cells produce both PDGF-A and B chains. The individual chains are assembled stochastically into disulfide-linked inactive homodimeric or heterodimeric precursors in the endoplasmic reticulum.[1 - 3] PDGF-A and -B isoforms were originally isolated from platelets, but were subsequently found to be produced by multiple cell types including megakaryocytes, fibroblasts, keratinocytes, vascular smooth muscle cells, endothelial cells, neurons, Schwann cells, and macrophages.[3] PDGF family proteins regulate diverse cellular functions by binding to and inducing the homo or heterodimerization of two receptor subunits (PDGF Rα and Rβ).[1 - 3] Both subunits belong to the class III subfamily of receptor tyrosine kinases. PDGF-BB can induce α/α or β/β homodimerization as well as α/β heterodimerization. PDGF plays important roles in development and regeneration. [4] During vascular development, endothelial cells secrete PDGF-BB, enhancing pericyte motility and recruitment.[5] Deletion of PDGF-B or PDGFRβ in mice leads to embryonic lethality due to lack of pericytes and/or VSMVs in blood vessels. PDGF-BB is frequently expressed at high levels in various tumor tissues where it promotes angiogenesis and metastasis synergistically with FGF-2,[6] likely through the involvement of the SDF-1α/CXCR4 axis.[7]

## References


1. Fredriksson, L. *et al.* (2004) Cytokine and Growth Factor Rev. **15**:197.
2. Li, X. & U. Eriksson (2003) Cytokine Growth Factor Rev. **14**:91.
3. Heldin, C-H. & B. Westermark (1999) Physiol. Rev. **79**:1283.
4. Hoch, R.V. & P. Soriano (2003) Development **130**:4769.
5. Hellstrom, M. *et al.* (1999) Development **126**:3047.
6. Nissen, L. J. (2007) J. Clin. Invest. **117**:2766.
7. Song, N. *et al.* (2009) Cancer Res. **69**:6057.


## Technical Notes

The human PDGF-BB biotin kit has been tested for its ability to stain human acute monocytic leukemia cell line THP-1 (Figure 1). Staining specificity has been determined by demonstrating a reduction in signal intensity when the staining reaction is carried out in the presence of 1 - 100 molar excess unconjugated PDGF-BB or in the presence of the blocking antibody. These inhibition reactions were carried out under limiting concentrations of PDGF-BB-biotin. Some cell lines can exhibit high non-specific staining with labeled cytokines. This effect can be compensated for by reducing the amount of labeled cytokine used in the reaction. We suggest that each user determine the optimal concentration of labeled cytokine by performing a dilution curve staining analysis on known receptor positive and negative cells. Normally, dilutions ranging from 1:2 to 1:10 are sufficient. Dilution of labeled cytokine should be made in 1X Cell Wash Buffer.

## Typical Data

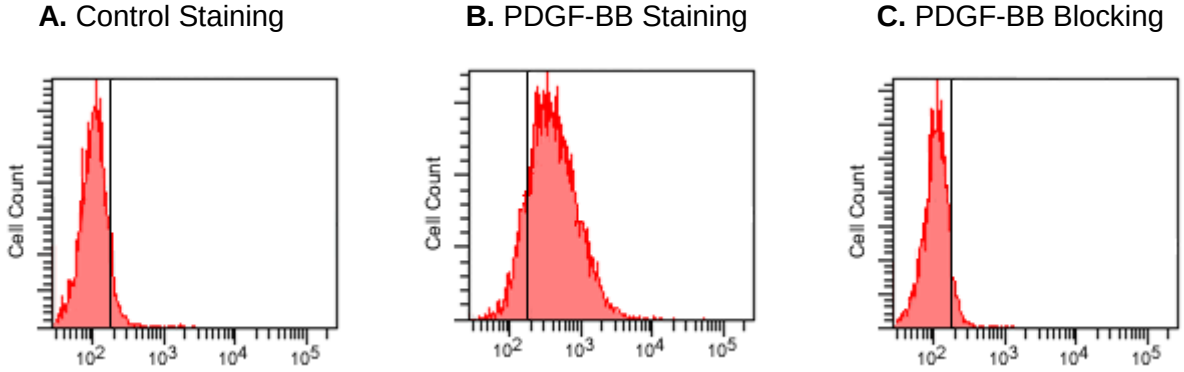


**Figure 1**. Human THP-1 cells were tested using the Biotinylated Human PDGF-BB Fluorokine Kit (Catalog # NFPDBB). Cells stained with the Negative Control protein (**A**) or with biotinylated recombinant human PDGF-BB (**B**) are shown. PDGF-BB staining can be specifically blocked with the anti-human PDGF-BB antibody (**C**). All reagents shown are provided with the kit.

**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.