# Fluorokine®
# Biotinylated Human Fractalkine

**Catalog Number:** NFCX310
**100 Tests**

## Reagents Provided

**Biotinylated rhFractalkine (100 reactions):** Lyophilized protein.

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

**Negative Control Reagent (60 reactions; 600 µL):** A protein (soybean trypsin inhibitor) biotinylated to the same degree as the studied protein.

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

**10X Cell Wash Buffer (60 mL):** 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 qualitatively and quantitatively determine the presence of cells expressing Fractalkine receptors or other interacting proteins by flow cytometry.

## Principle of the Test

Washed cells are incubated with the biotinylated protein that in turn binds to the cells via specific cell surface receptors or other interacting proteins. The cells are then directly incubated with avidin-fluorescein, which attaches to the bound biotinylated protein. Cells expressing the receptor interacting protein are fluorescently stained, with the intensity of staining proportional to the density of such protein. Relative density is then determined by flow cytometric analysis using 488 nm wavelength laser excitation.

## Reagent Preparation

**Biotinylated rhFractalkine:** Add 1 mL of sterile distilled water to the lyophilized product. Mix gently until the product is completely dissolved. **Reagent yields optimal activity when used within 60 days of reconstitution.**

**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 pre-treatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from the substrate 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 protein 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 can be pre-treated 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 Fractalkine blocking antibody is mixed with 10 µL of Fractalkine-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-Fractalkine 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

CX3CL1, also named neurotactin, is a novel chemokine identified through bioinformatics. CX3CL1 has a unique C-X3-C cysteine motif near the amino-terminus and is the first member of a fourth branch of the chemokine superfamily. Unlike other known chemokines, CX3CL1 is a type 1 membrane protein containing a chemokine domain tethered on a long mucin-like stalk. Human CX3CL1 cDNA encodes a 397 amino acid (aa) residue membrane protein with a 24 aa residue predicted signal peptide, a 76 aa residue chemokine domain, a 241 aa residue stalk region containing 17 degenerate mucinlike repeats, a 19 aa residue transmembrane segment and a 37 aa residue cytoplasmic domain. The extracellular domain of human CX3CL1 can be released, possibly by proteolysis at the dibasic cleavage site proximal to the membrane, to generate soluble CX3CL1. CX3CL1 mRNA has been detected in various tissues including the brain and heart. The expression of CX3CL1 was also reported to be upregulated in endothelial cells and microglia by inflammatory signals. Membrane-bound CX3CL1 has been shown to promote adhesion of leukocytes. The soluble chemokine domain of human CX3CL1 was reported to be chemotactic for T cells and monocytes while the soluble chemokine domain of mouse CX3CL1 was reported to chemoattract neutrophils and T lymphocytes but not monocytes. The gene for human CX3CL1 has been mapped to chromosome 16q.

## References


1. Pan, Y. *et al*. (1997) Nature **387**:611.
2. Bazan J.F. *et al*. (1997) Nature **385**:640.
3. Mackay, C.R. (1997) Current Biology **7**:R384.


## Technical Notes

Human Fractalkine biotin kit has been tested for its ability to stain human acute monocytic leukemia THP-1 cell line **(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 a specific anti-Fractalkine blocking antibody. These inhibition reactions were carried out under limiting concentrations of Fractalkine-biotin. Some cell lines can exhibit high non-specific staining with labeled proteins. This effect can be compensated for by reducing the amount of labeled protein used in the reaction. We suggest that each user determine the optimal concentration of labeled protein 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 protein should be made in 1X Cell Wash Buffer.

## Typical Data

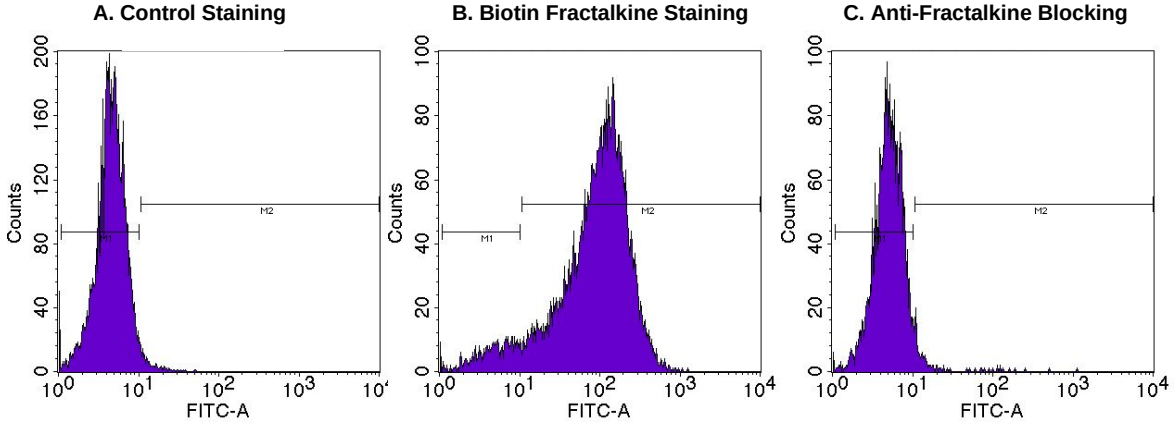


**Figure 1.** Human THP-1 cells were tested using the Fractalkine-biotin kit (NFCX310). Cells stained with **(A)** the Negative Control protein or **(B)** rhFractalkine-biotin are shown. Fractalkine staining can be specifically blocked with **(C)** anti-human Fractalkine antibody. Fluorescein-conjugated Avidin was used as secondary stain. 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.