# Fluorokine®
# Biotinylated Human VEGF

**Catalog Number:** NFVE0
**100 Tests**

## Reagents Provided

**Biotinylated rhVEGF (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 VEGF 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 VEGF 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 rhVEGF:** 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 VEGF blocking antibody is mixed with 10 µL of VEGF-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-VEGF 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

Vascular endothelial growth factor (VEGF or VEGF-A), also known as vascular permeability factor (VPF), is a potent mediator of both angiogenesis and vasculogenesis in the fetus and adult (1-3). It is a member of the PDGF family that is characterized by the presence of eight conserved cysteine residues and a cystine knot structure (4). Humans express alternately spliced isoforms of 121, 145, 165, 183, 189, and 206 amino acids (aa) in length (4). $VEGF_{165}$ appears to be the most abundant and potent isoform, followed by $VEGF_{121}$ and $VEGF_{189}$ (3, 4). Isoforms other than $VEGF_{121}$ contain basic heparin-binding regions and are not freely diffusible (4). Human VEGF165 shares 88% aa sequence identity with corresponding regions of mouse and rat, 96% with porcine, 95% with canine, and 93% with feline, equine and bovine VEGF, respectively. VEGF binds the type I transmembrane receptor tyrosine kinases VEGF R1 (also called Flt1) and VEGF R2 (Flk-1/ KDR) on endothelial cells (4). Although VEGF affinity is highest for binding to VEGF R1, VEGF R2 appears to be the primary mediator of VEGF angiogenic activity (3, 4). VEGF165 binds the semaphorin receptor, Neuropilin-1 and promotes complex formation with VEGF R2 (5). VEGF is required during embryogenesis to regulate the proliferation, migration, and survival of endothelial cells (3, 4). In adults, VEGF functions mainly in wound healing and the female reproductive cycle (3). Pathologically, it is involved in tumor angiogenesis and vascular leakage (6, 7). Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (8). VEGF is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, Oncostatin M, and TNF-$\alpha$.

## References


1. Leung, D.W. *et al.* (1989) Science **246**:1306.
2. Keck, P.J. *et al.* (1989) Science **246**:1309.
3. Byrne, A.M. *et al.* (2005) J. Cell. Mol. Med. **9**:777.
4. Robinson, C.J. and S.E. Stringer (2001) J. Cell. Sci. **114**:853.
5. Pan, Q. *et al.* (2007) J. Biol. Chem. **282**:24049.
6. Weis, S.M. and D.A. Cheresh (2005) Nature **437**:497.
7. Thurston, G. (2002) J. Anat. **200**:575.
8. Carvalho, J.F. *et al.* (2007) J. Clin. Immunol. **27**:246.
9. Angelo, L.S. and R. Kurzrock (2007) Clin. Cancer Res. **13**:2825.


## Technical Notes

Human VEGF biotin kit has been tested for its ability to stain human acute T cell leukemia Jurkat 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-VEGF blocking antibody. These inhibition reactions were carried out under limiting concentrations of VEGF-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

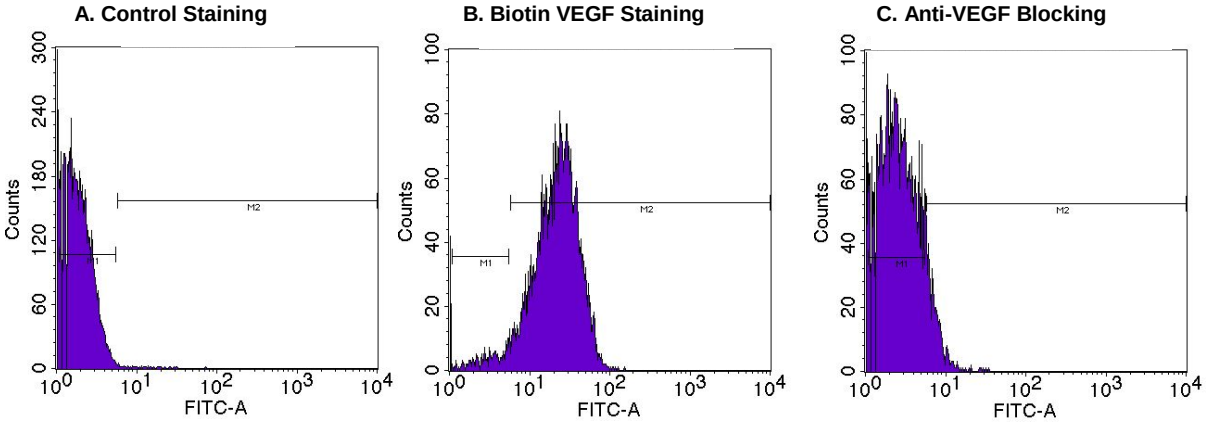


**Figure 1.** Human Jurkat cells were tested using this VEGF-biotin kit. Cells stained with **(A)** the Negative Control protein or **(B)** rhVEGF-biotin are shown. VEGF staining can be specifically blocked with **(C)** anti-human VEGF antibody. Fluorescein-conjugated Avidin was used as secondary stain.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