

# **Anti-VEGF Receptor 3/FLT4 Antibody Picoband™**

Catalog Number: A01276-2

#### **About FLT4**

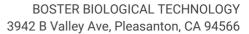
Fms-related tyrosine kinase 4, also known as FLT4 or VEGFR3, is a protein which in humans is encoded by the FLT4 gene. It is mapped to 5q35.3. This gene encodes a tyrosine kinase receptor for vascular endothelial growth factors C and D. The protein is thought to be involved in lymphangiogenesis and maintenance of the lymphatic endothelium. FLT4 has an essential role in the development of the embryonic cardiovascular system before the emergence of the lymphatic vessels. It has been found that FLT4, which provides proangiogenic signaling when expressed on endothelium, may also have antiangiogenic properties when expressed at an avascular site by nonendothelial cells. FLT4 is also regarded as a regulator of vascular network formation.

#### Overview

Product Name	Anti-VEGF Receptor 3/FLT4 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-VEGF Receptor 3/FLT4 Antibody Picoband™ catalog # A01276-2. Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	FLT4: P35916

#### **Technical Details**

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human VEGF Receptor 3, which shares 82.8% and 79.3% amino acid (aa) sequence identity with mouse and rat VEGF Receptor 3, respectively.
Predicted Reactive Species	Human
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG





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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.  If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.  Some PubMed article(s) citing the expression level of this target are as follows:  Boster Bio's internal QC testing used:  Western blot, 0.1-0.5ug/ml  Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml  Immunohistochemistry (Frozen Section), 0.5-1ug/ml  Immunocytochemistry/Immunofluorescence, 2ug/ml  Flow Cytometry, 1-3ug/1x10 <sup>6</sup> cells



### Anti-VEGF Receptor 3/FLT4 Antibody Picoband™ (A01276-2) Images

as a control.

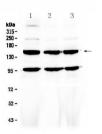


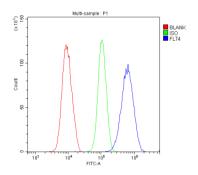
Figure 1. Western blot analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,

Lane 2: human MCF-7 whole cell lysates,

Lane 3: human HepG2 whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-VEGF Receptor 3 antigen affinity purified polyclonal antibody (Catalog # A01276-2) at 0.5 ug/mL overnight at 4 then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for VEGF Receptor 3 at approximately 153KD. The expected band size for VEGF Receptor 3 is at 153KD.



VEGF Receptor 3 antibody (A01276-2). Overlay histogram showing U20S cells stained with A01276-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-VEGF Receptor 3 Antibody (A01276-2,1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used

Figure 10. Flow Cytometry analysis of U20S cells using anti-

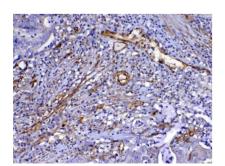


Figure 2. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



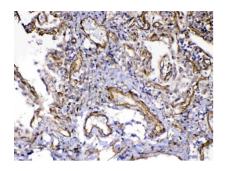


Figure 3. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

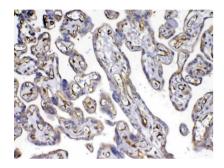


Figure 4. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

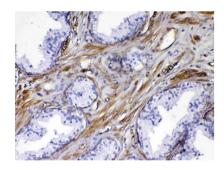


Figure 5. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of human prostatic cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

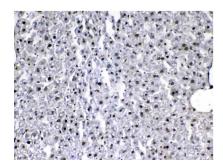


Figure 6. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of mouse liver tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



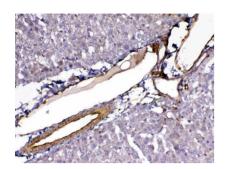


Figure 7. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of rat liver tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

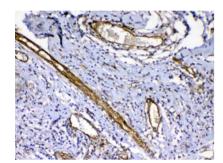


Figure 8. IHC analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4 Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

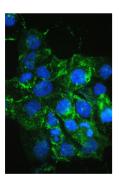


Figure 9. IF analysis of VEGF Receptor 3 using anti-VEGF Receptor 3 antibody (A01276-2).

VEGF Receptor 3 was detected in immunocytochemical section of A431 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-VEGF Receptor 3 Antibody (A01276-2) overnight at 4°C. DyLight® 488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

## 2 Publications Citing This Product

1. PubMed ID: 16961284, Serum vascular endothelial growth factor-C and vascular endothelial growth factor level in patients with colorectal carcinoma and clinical significance

2. PubMed ID: 24040410, Li C, Fan J, Song X, Zhang B, Chen Y, Li C, Mi K, Ma H, Song Y, Tao X, Li G. Plos One. 2013 Sep 11;8(9):E75388. Doi: 10.1371/Journal.Pone.0075388. Ecollection 2013. Expression Of Angiopoietin-2 And Vascular Endothelial Growth Factor Receptor-3 Cor...

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