

Product Datasheet

VEGFR1/Flt-1 Antibody - BSA Free NB100-527SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB100-527SS

VEGFR1/Flt-1 Antibody - BSA Free

Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

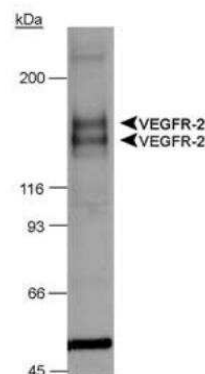
Product Description	
Host	Rabbit
Gene ID	2321
Gene Symbol	FLT1
Species	Human
Marker	Endothelial Cell Marker
Specificity/Sensitivity	This antibody targets VEGFR-1 but has significant cross-reactivity with the VEGFR-2 protein.
Immunogen	A synthetic peptide made to an internal region of the mouse VEGF Receptor 1 protein (between residues 800-900) [Uniprot: P35969]. There is 87% identity between the immunogen used for this production and the VEGF Receptor 2 protein.

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:100 - 1:2000, Immunohistochemistry 1:250 - 1:500, Immunocytochemistry/ Immunofluorescence 1:500, Immunohistochemistry-Paraffin 1:250 - 1:500

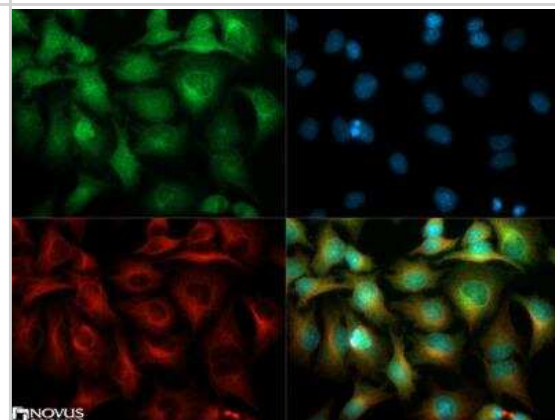


Images

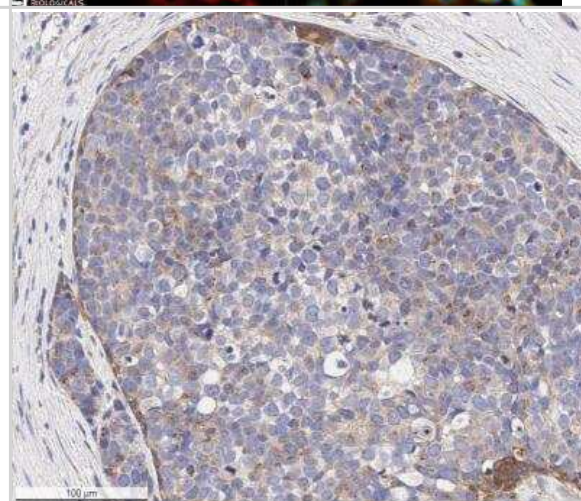
Western Blot: VEGF R1/Flt-1 Antibody [NB100-527] - Chimeric CSF-1R/VEGFR-2 detection in transfected lysates.



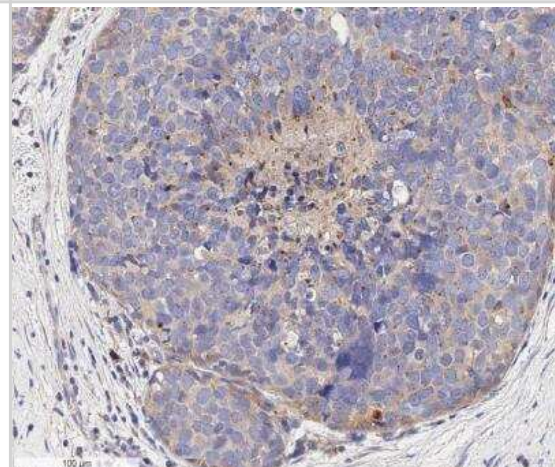
Immunocytochemistry/Immunofluorescence: VEGFR1/Flt-1 Antibody [NB100-527] - VEGF R1/Flt-1 antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



Immunohistochemistry-Paraffin: VEGFR1/Flt-1 Antibody [NB100-527] - Analysis of FFPE human breast carcinoma tissue section using 1:500 dilution of VEGFR1/Flt-1 antibody on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) with 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching using peroxide block. The sections were incubated with primary antibody for 30 minutes. Bond Polymer Refine Detection (Leica Biosystems) and DAB were used for signal detection which followed counterstaining with hematoxylin. Whole slide scanning and capturing of representative images (20X) were performed using Aperio AT2 (Leica Biosystems). This VEGFR1/Flt-1 antibody generated an expected membrane cytoplasmic staining of VEGFR1 protein in the cancer cells (punctate appearance typical of receptors). The tumor stroma/stromal cells did not show VEGFR1/Flt-1 immunopositivity.



Immunohistochemistry-Paraffin: VEGFR1/Flt-1 Antibody [NB100-527] - Analysis of a FFPE human breast carcinoma tissue section using 1:500 dilution of VEGFR1/Flt-1 antibody on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) with 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching using peroxide block. The sections were incubated with primary antibody for 30 minutes. Bond Polymer Refine Detection (Leica Biosystems) and DAB were used for signal detection which followed counterstaining with hematoxylin. Whole slide scanning and capturing of representative images (20X) were performed using Aperio AT2 (Leica Biosystems). This VEGFR1/Flt-1 antibody generated an expected membrane cytoplasmic staining of VEGFR1 protein in the cancer cells. The tumor stroma/stromal cells did not show VEGFR1/Flt-1 immunopositivity. Staining was performed by Histowiz.



Publications

Podemska-Jedrzejczak Z, Malinska A, et al. Vascular restenosis in coronary artery bypass grafting might be associated with VEGF-C/VEGFR-3 signaling pathway. *Heart Vessels* 2018-09-01 [PMID: 29557990] (IF/IHC, Human)

Nascimento JJAC, Machado ASD, Della-Santa GML et al. Effects of photobiomodulation therapy on functional recovery, angiogenesis and redox status in denervated muscle of rats Einstein (Sao Paulo, Brazil) 2021-09-13 [PMID: 34586157] (IF/IHC, Rat)

Su CC. Tanshinone IIA can inhibit MiaPaCa 2 human pancreatic cancer cells by dual blockade of the Ras/Raf/MEK/ERK and PI3K/AKT/mTOR pathways. *Oncol. Rep.* 2018-11-01 [PMID: 30226540] (WB, Human)

Ptaszynska MM, Pendrak ML, Bandle RW, Stracke ML, Roberts DD. Positive feedback between vascular endothelial growth factor-A and autotaxin in ovarian cancer cells. *Mol Cancer Res*;6(3):352-63. 2008-03-01 [PMID: 18337445] (WB, Human)

Puglisi F, Puppini C, Pegolo E et al. Expression of periostin in human breast cancer. *J Clin Pathol*;614:494-8. 2008-04-01 [PMID: 17938160]



Procedures

Western Blot Protocol for VEGFR1/Flt-1 Antibody (NB100-527)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for VEGFR1/Flt-1 Antibody (NB100-527)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.



Immunohistochemistry-Paraffin Protocol for VEGFR1/Flt-1 Antibody (NB100-527)**Immunohistochemistry-Paraffin Embedded Sections****Antigen Unmasking:**

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.





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