Product Datasheet

VEGFR2/KDR/Flk-1 Antibody
NB100-2382

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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Publications: 5

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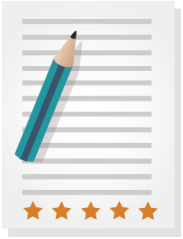
Updated 1/29/2020 v.20.1



NB100-2382

VEGFR2/KDR/Flk-1 Antibody

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)
Target Molecular Weight	150 kDa

Product Description	
Host	Rabbit
Gene ID	3791
Gene Symbol	KDR
Species	Human, Rat, Hamster
Reactivity Notes	Human. Predicted to react with mouse based on 100% sequence homology. Immunogen sequence has 79% homology to rat. Hamster reactivity reported in scientific literature (PMID: 23763710). Rat reactivity reported in scientific literature (PMID: 27832737).
Marker	Endothelial Cell Marker
Specificity/Sensitivity	This has only been shown to recognize the native form (180-190 kDa protein) of VEGFR-2, known as Flk-1/KDR/VEGFR 2 (Vascular Endothelial Growth Factor Receptor 2), though it was raised against the precursor form.
Immunogen	A synthetic peptide made to a C-terminal region of the mouse VEGF Receptor 2 protein (between residues 1300-1367). [Swiss-Prot# P35918]

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence
Recommended Dilutions	Western Blot 1 ug/ml, Flow Cytometry, Immunocytochemistry/Immunofluorescence 1:10
Application Notes	This VEGF Receptor 2 antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot, where a doublet is seen at ~150 kDa with CSF-1R/VEGFR2 chimera transfected lysates representing VEGFR-2. A non-specific band is also seen at ~85 kDa. In ICC/IF punctate membrane staining was observed in Hela cells. Use in FLOW cytometry reported in scientific literature (PMID 27832737).

Publications

Roan, J N, Cheng, H N Et al. Exendin-4, a glucagon-like peptide-1 analogue, accelerates diabetic wound healing. J Surg Res 2017-02-01 [PMID: 27993221] (IF/IHC, Mouse)

Zhou J, Rogers J, Lee S et al. Oral mucosa harbors a high frequency of endothelial cells - a novel postnatal cell source for angiogenic regeneration. Stem Cells Dev. 2016-11-10 [PMID: 27832737] (FLOW, Rat)

Details:
This citation used the DyLight 488 version of this antibody.

Roan JN, Fang SY, Chang SW et al. Rosuvastatin improves vascular function of arteriovenous fistula in a diabetic rat model. J Vasc Surg 2012-06-22 [PMID: 22727844] (FLOW, Rat)

Details:
Using the DyLight 488 conjugated version of NB100-2382, catalog number NB100-2382G.

Lim UM, Yap MG, Lim YP et al. Identification of Autocrine Growth Factors Secreted by CHO Cells for Applications in Single-Cell Cloning Media. J Proteome Res 2013-07-05 [PMID: 23763710] (WB, Hamster)

Hu-Lowe DD et al. Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. Clin Cancer Res;14(22):7272-83. 2008-11-15 [PMID: 19010843]

Procedures

Western blot protocol specific for VEGF Receptor 2 Antibody (NB100-2382)
Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. Rinse membrane with dH2O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

4. Rinse the blot in TBS for approximately 5 minutes.

5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS overnight at 4 degrees Celsius.

6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.

7. Dilute the rabbit anti-VEGFR2 primary antibody (NB 100-2382) in blocking buffer and incubate 1 hour at room temperature.

8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.

9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.

10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 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 (we used BioFX Super Plus ECL).

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

ICC/IF protocol specific for VEGF Receptor 2 Antibody (NB100-2382)
Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.
5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.
6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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