

Product Datasheet

VEGFR2/KDR/FIk-1 Antibody - BSA Free NB100-530

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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NB100-530

VEGFR2/KDR/FIk-1 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	150 kDa

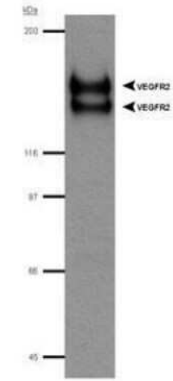
Product Description	
Description	Novus Biologicals Rabbit VEGFR2/KDR/FIk-1 Antibody - BSA Free (NB100-530) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-VEGFR2/KDR/FIk-1 Antibody: Cited in 4 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	3791
Gene Symbol	KDR
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 28183816).
Marker	Endothelial Cell Marker
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, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:250-1:1000, Flow Cytometry reported in scientific literature (PMID 29873146), Immunohistochemistry reported in scientific literature (PMID 30941660), Immunocytochemistry/ Immunofluorescence 1:50 - 1:500, Flow (Intracellular)
Application Notes	In Western blot a doublet is seen at ~150 kDa. In ICC/IF nuclear and membrane staining can be seen in V6.5 mouse embryonic stem cells (Catalog No. NBP1-41162). The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

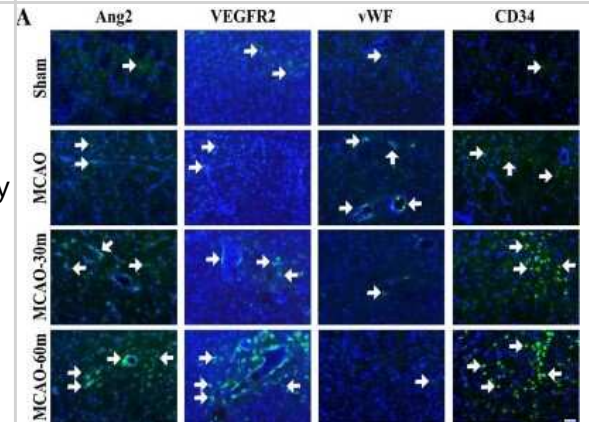


Images

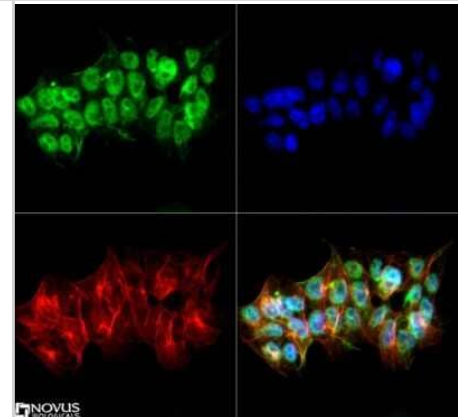
Western Blot: VEGFR2/KDR/Fik-1 Antibody [NB100-530] - Detection of VEGF R2/KDR/Fik-1 doublet in CSF-1 receptor/VEGF R2/KDR/Fik-1 chimera transfected lysate (20 ug) using NB100-530 (0.5 ug/mL). ECL detection 10 seconds.



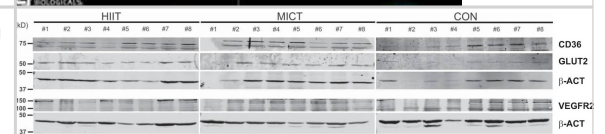
Immunocytochemistry/Immunofluorescence: VEGFR2/KDR/Fik-1 Antibody [NB100-530] - Immunohistochemical analyses of VEGFR2, Ang-2, vWF and CD34 in the peri-infarct area of the ischemic brain. Representative merged images counterstained with DAPI (nuclear staining) (blue). Ang-2, VEGFR2, vWF and CD34 antibodies are represented by the green color (arrows). Image collected and cropped by Citeab from the following publication (A Short Bout of Exercise Prior to Stroke Improves Functional Outcomes by Enhancing Angiogenesis. *Neuromolecular Med* (2019) licensed under a CC-BY license.



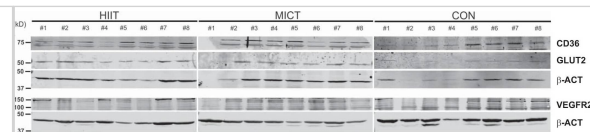
Immunocytochemistry/Immunofluorescence: VEGFR2/KDR/Fik-1 Antibody [NB100-530] - V6.5 mouse embryonic stem cells (cat# NBP1-41162) stained with Dylight 488 (Green). Alpha-tubulin and nuclei were counterstained against Dylight 550 (Red) and DAPI (Blue).



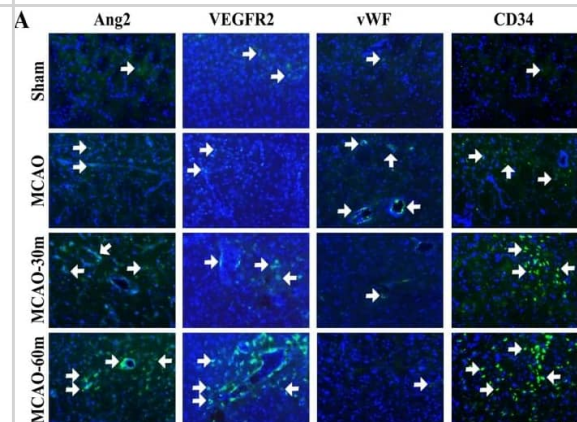
Top: relative expression of CD36, GLUT2, and VEGFR2 in duodenum; n = 6–8. All values are expressed as model-based means, with error bars representing the confidence intervals (95% CI). *P value < 0.05. Bottom: Western blots of CD36 (75 kDa), GLUT2 (55 kDa), and VEGFR2 (105 kDa). Animals without a detectable band were excluded from the analysis. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; CON, control group.



Western Blot: VEGFR2/KDR/FIk-1 Antibody [NB100-530] - Top: relative expression of CD36, GLUT2, & VEGFR2 in duodenum; n = 6–8. All values are expressed as model-based means, with error bars representing the confidence intervals (95% CI). *P value < 0.05. Bottom: Western blots of CD36 (75 kDa), GLUT2 (55 kDa), & VEGFR2 (105 kDa). Animals without a detectable band were excluded from the analysis. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; CON, control group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28183816>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemical analyses of VEGFR2, Ang-2, vWF and CD34 in the peri-infarct area of the ischemic brain. a Representative merged images counterstained with DAPI (nuclear staining) (blue). Ang-2, VEGFR2, vWF and CD34 antibodies are represented by the green color (arrows). b Relative fluorescence intensity showed that Ang-2, VEGFR2, and CD34 were increased in the exercise groups. After exercise, vWF was decreased. No significant differences were observed between groups ($p > 0.05$). Sham: (n = 4); MCAO: (n = 4); MCAO-30m: (n = 4); MCAO-60m: (n = 4). Bar = 50 μ m. Error bars represent S.E.M Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/30941660>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Liu X, Yu Z, Daitoku K et al. Human aortic valve interstitial cells obtained from patients with aortic valve stenosis are vascular endothelial growth factor receptor 2 positive and contribute to ectopic calcification *Journal of Pharmacological Sciences* 2021-02-01 [PMID: 33451756] (ICC/IF, Human)

Pianta, S;Lee, JY;Tuazon, JP;Castelli, V;Mantohac, LM;Tajiri, N;Borlongan, CV; A Short Bout of Exercise Prior to Stroke Improves Functional Outcomes by Enhancing Angiogenesis *Neuromolecular Med.* 2019-04-02 [PMID: 30941660] (IF/IHC, Rat)

Qureshi R, Kindo M, Boulberdaa M et al. A prokineticin-driven epigenetic switch regulates human epicardial cell stemness and fate *Stem Cells* 2018-06-06 [PMID: 29873146] (FLOW, Human)

Motiani KK, Savolainen AM, Eskelinen JJ et al. Two weeks of moderate intensity continuous training, but not high intensity interval training increases insulin-stimulated intestinal glucose uptake. *J. Appl. Physiol.* 2017-02-09 [PMID: 28183816] (WB, Rat)

Procedures

Western Blot Protocol for VEGF Receptor 2 Antibody (NB100-530)

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 VEGFR2/KDR/Fik-1 Antibody (NB100-530)

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. Permeablize 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 VEGFR2/KDR/Flk-1 Antibody (NB100-530)

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.



Flow (Intracellular) Protocol for VEGFR2/KDR/FIk-1 Antibody (NB100-530)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 μ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 μ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 μ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.



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Products Related to NB100-530

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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