Product Datasheet

VEGF R2/KDR/Flk-1 Antibody

NB100-530SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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Updated 6/15/2014 v.20.1

NB100-530SS

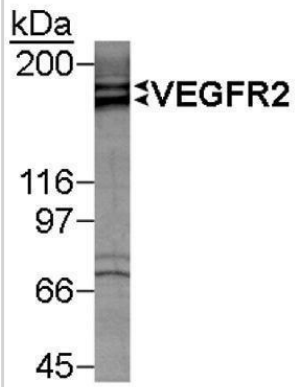
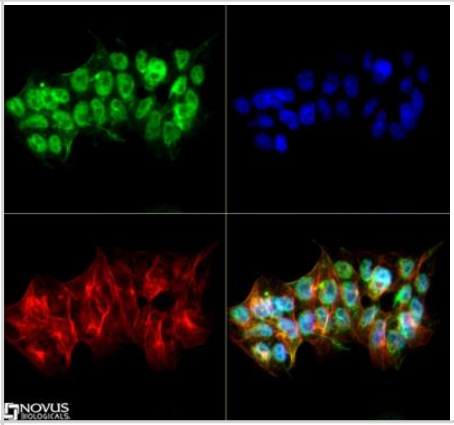
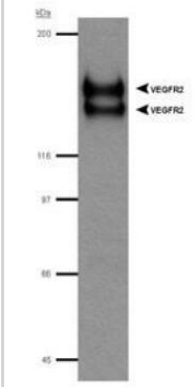
VEGF R2/KDR/Flk-1 Antibody

Product Information	
Unit Size	0.025 ml
Concentration	2.55 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-glycine, 150 mM NaCl
Target Molecular Weight	150 kDa

Product Description	
Host	Rabbit
Gene ID	3791
Gene Symbol	KDR
Species	Human, Mouse
Species Reactivity	Human and mouse. Predicted to react with rat based on 100% sequence homology.
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, Immunocytochemistry/Immunofluorescence
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:50-1:500, Western Blot 1:250-1:1000
Application Notes	This VEGF Receptor 2 antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot, where 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).

Images

Western Blot: VEGF R2/KDR/Flk-1 Antibody [NB100-530] - Detection of VEGF R2/KDR/Flk-1 doublet in VEGF R2/KDR/Flk-1 induced HUVEC lysate (50 ug) using NB100-530 (1 ug/ml). ECL detection 1 minute.	
Immunocytochemistry/Immunofluorescence: VEGF R2/KDR/Flk-1 Antibody [NB100-530] - The VEGF R2/KDR/Flk-1 antibody was tested in V6.5 mouse embryonic stem cells (cat# NBP1-41162) against Dylight 488 (Green). Alpha-tubulin and nuclei were counterstained against Dylight 550 (Red) and DAPI (Blue), respectively.	
Western Blot: VEGF R2/KDR/Flk-1 Antibody [NB100-530] - Detection of VEGF R2/KDR/Flk-1 doublet in CSF-1 receptor/VEGF R2/KDR/Flk-1 chimera transfected lysate (20 ug) using NB100-530 (0.5 ug/ml). ECL detection 10 seconds.	

Procedures

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

1. Perform SDS-PAGE (3-8%) on samples to be analyzed, loading 50 ug of total protein per lane.

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

3. 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 + 0.5% BSA in TBS for 1 hour.

6. Dilute the rabbit anti-VEGFR2 primary antibody (NB 100-530) in blocking buffer and incubate 2 hours at room temperature.

7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.

8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.

9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).

10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

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

Immunocytochemistry/Immunofluorescence Protocol for VEGF Receptor 2 Antibody (NB100-530)
Immunocytochemistry Protocol

Culture cells to appropriate density 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 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.