

# Product Datasheet

## ROBO4 Antibody - BSA Free NB110-58778

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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**NB110-58778**

ROBO4 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.09 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)

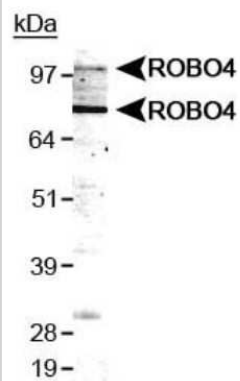
Product Description	
Description	Novus Biologicals Rabbit ROBO4 Antibody - BSA Free (NB110-58778) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	54538
Gene Symbol	ROBO4
Species	Human
Marker	Tumor Endothelial Marker
Specificity/Sensitivity	This recognizes the 85 kDa and 107 kDa isoforms of the ROBO4 human protein.
Immunogen	A synthetic peptide made to an internal region of human Robo4 (within residues 200-300). [Swiss-Prot# Q8WZ75]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:500, Immunohistochemistry 5 ug/ml, Immunocytochemistry/Immunofluorescence, Immunohistochemistry-Paraffin 5 ug/ml
Application Notes	This Robo4 antibody is useful for Immunohistochemistry-Paraffin and Western Blot analysis, where bands at 85 kDa and 107 kDa are observed representing different isoforms of ROBO4 protein.

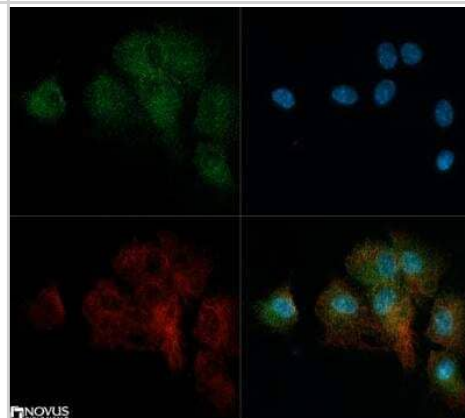


## Images

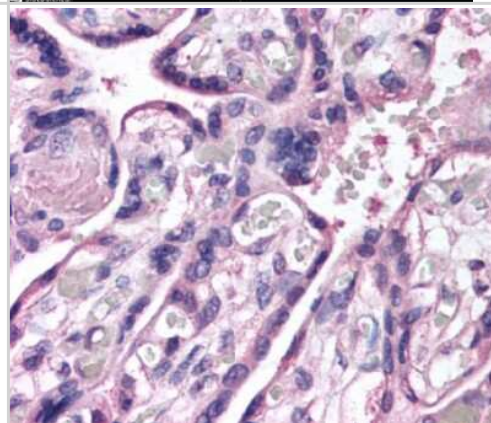
Western Blot: Robo4 Antibody [NB110-58778] - Detection of ROBO4 in HUVEC lysate



Immunocytochemistry/Immunofluorescence: Robo4 Antibody [NB110-58778] - Robo4 antibody was tested in Huvec cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Immunohistochemistry: Robo4 Antibody [NB110-58778] - Detection of ROBO4 in endothelium and trophoblasts of the placenta.



## Procedures

### Serum protocol for ROBO4 Antibody (NB110-58778)

ROBO4 Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 28 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH<sub>2</sub>O 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, 1 hour at room temperature.
6. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-ROBO4 primary antibody (NB 110-58778) in blocking buffer and incubate 2 hours at room temperature.
8. Rinse the membrane in dH<sub>2</sub>O 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 manufacturers 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 manufacturers instructions (Pierce, 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.

### Immunocytochemistry/Immunofluorescence protocol for ROBO4 Antibody (NB110-58778)

ROBO4 Antibody:

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 1:25,000 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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### **Products Related to NB110-58778**

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NBL1-15473	ROBO4 Overexpression Lysate
NB110-58778PEP	ROBO4 Antibody Blocking Peptide
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

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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.

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