

# Product Datasheet

## Numb Antibody - BSA Free

### NB500-178

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NB500-178**

Numb Antibody - BSA Free

**Product Information**

<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	2.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	Tris-Glycine and 0.15M NaCl

**Product Description**

<b>Host</b>	Rabbit
<b>Gene ID</b>	8650
<b>Gene Symbol</b>	NUMB
<b>Species</b>	Human, Mouse, Chicken
<b>Reactivity Notes</b>	Immunogen sequence has 90% identity to rat.
<b>Specificity/Sensitivity</b>	This is specific for all four isoforms of the NUMB protein.
<b>Immunogen</b>	A synthetic peptide made to a C-terminal region of mouse NUMB (between residues 600-653). [UniProt# Q9QZS3]

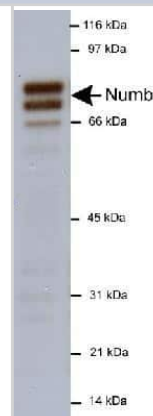
**Product Application Details**

<b>Applications</b>	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence
<b>Recommended Dilutions</b>	Western Blot 0.2-0.5 ug/ml, Simple Western 1:200, Immunocytochemistry/ Immunofluorescence 1:500
<b>Application Notes</b>	<p>In WB one may see any or all of the four isoforms. The molecular weight of human and mouse NUMB is 72 kDa (isoform 1), 66 kDa (isoform 2), 71 kDa (isoform 3), and 65 kDa (isoform 4). Multiple non-specific bands may be seen with lower dilutions and/or longer exposure times than suggested in the protocol we used to obtain our data.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in A431 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:200, apparent MW was 83 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

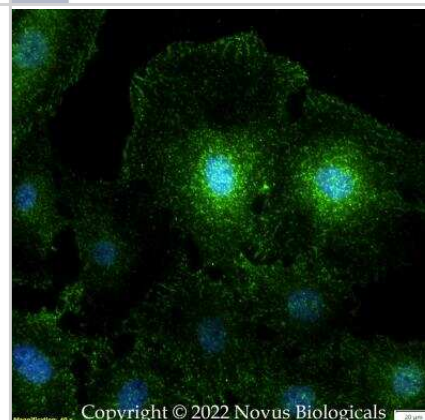


## Images

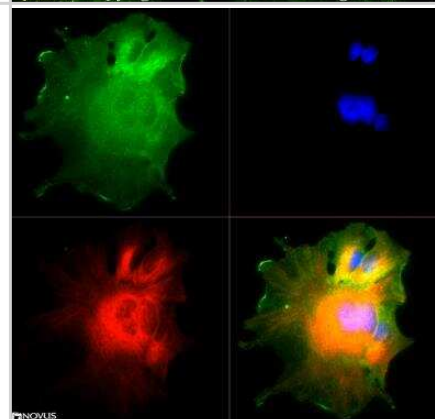
Western Blot: NUMB Antibody [NB500-178] - Detection of NUMB isoforms 1 and 2 in A431 whole cell lysate (20 ug) using 0.5 ug/ml of NB500-178. ECL detection: 30 seconds.



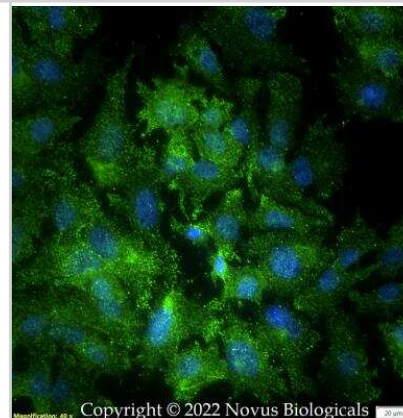
Immunocytochemistry/Immunofluorescence: Numb Antibody [NB500-178] - Rat FR cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with (NB500-178) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunocytochemistry/Immunofluorescence: NUMB Antibody [NB500-178] - The NUMB antibody was tested in HepG2 cells at a 1:500 dilution against Dylight 488 (Green). Alpha-tubulin and nuclei were counterstained against Dylight 550 (Red) and DAPI (Blue), respectively.



Immunocytochemistry/Immunofluorescence: Numb Antibody [NB500-178] - HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Numb Antibody (NB500-178) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Simple Western: Numb Antibody [NB500-178] - Simple Western lane view shows a specific band for NUMB in 0.5 mg/ml of A431 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



## Publications

Das A, Adhikary S, Chowdhury AR, Barui A Chirality-induced Lineage Enforcement of Mechanosensitive Mesenchymal Stem Cells Across Germ Layer Boundaries Stem cell reviews and reports 2023-11-16 [PMID: 37971671]

Das A, Adhikary S, Chowdhury AR Et al. Leveraging Substrate Stiffness to Promote Stem Cell Asymmetric Division via Mechanotransduction-Polarity Protein Axis and Its Bayesian Regression Analysis Rejuvenation Res 2022-03-22 [PMID: 35316074] (ICC/IF)

### Details:

Citation using the Texas Red version of this antibody.

Huang SC, Vu LV, Yu FH Et al. Multifunctional protein 4.1R regulates the asymmetric segregation of Numb during terminal erythroid maturation The Journal of biological chemistry 2021-08-06 [PMID: 34364872]

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

### Details:

Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Lenihan JA, Saha O, Young PW et al. Proteomic analysis reveals novel ligands and substrates for LNX1 E3 ubiquitin ligase PLoS One. 2017-11-08 [PMID: 29121065] (WB, Human)

Wang H, Xiang D, Liu B et al. Inadequate DNA Damage Repair Promotes Mammary Transdifferentiation, Leading to BRCA1 Breast Cancer Cell 2019-06-27 [PMID: 31251913] (Mouse)

Lenihan JA, Saha O, Heimer-McGinn V et al. Decreased Anxiety-Related Behaviour but Apparently Unperturbed NUMB Function in Ligand of NUMB Protein-X (LNX) 1/2 Double Knockout Mice. Mol. Neurobiol. 2016-11-26 [PMID: 27889896] (WB, Mouse)

## Procedures

### Western Blot protocol for Numb Antibody (NB500-178)

Numb Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 20ug 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 in TBS for 1 hour.
6. Dilute the rabbit anti-Numb primary antibody (NB 500-178) 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.





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### **Products Related to NB500-178**

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NB820-59461	A-431 Whole Cell Lysate
NB500-178PEP	Numb Antibody Blocking Peptide
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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