

Product Datasheet

AMBRA1 Antibody - BSA Free NBP1-07124

Unit Size: 0.2 ml

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

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NBP1-07124

AMBRA1 Antibody - BSA Free

Product Information	
Unit Size	0.2 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol

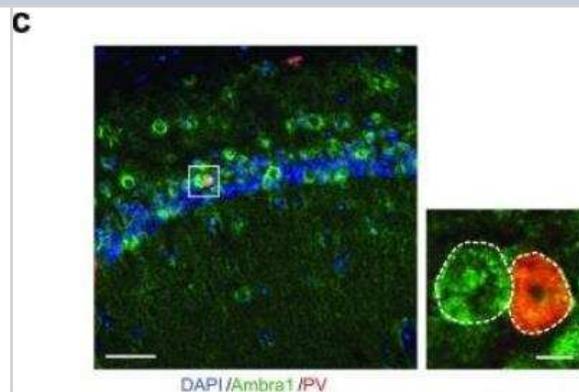
Product Description	
Description	Novus Biologicals Rabbit AMBRA1 Antibody - BSA Free (NBP1-07124) is a polyclonal antibody validated for use in IHC, WB, ELISA and ICC/IF. Anti-AMBRA1 Antibody: Cited in 6 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	55626
Gene Symbol	AMBRA1
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide derived from mouse activating molecule in Beclin 1-regulated/AMBRA1 protein sequence [amino acids range 1175-1250] [UniProt # A2AH22]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, ELISA, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:1000, ELISA 1:100-1:2000, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:400
Application Notes	This AMBRA1 antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence, and IHC-paraffin embedded sections. In Western Blot, a band is seen ~ 142 kDa representing AMBRA1. In ICC/IF, cytoplasmic staining was observed in HeLa cells. In IHC-P, staining was observed compartmentalized in cytoplasmic vesicles of mouse brain and skin tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

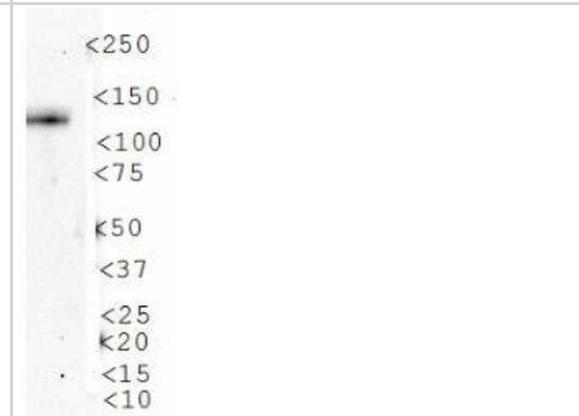


Images

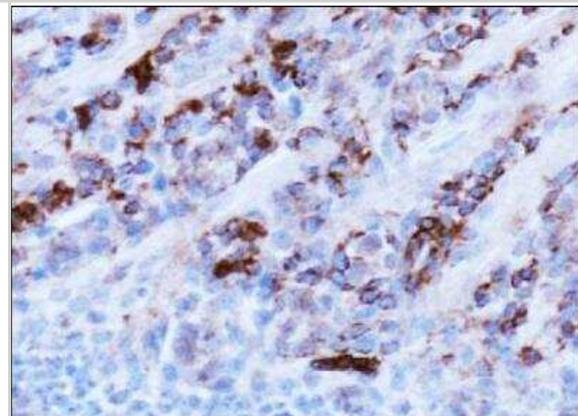
Immunocytochemistry/Immunofluorescence: AMBRA1 Antibody [NBP1-07124] - Double labeling for Ambra1 (green) and PV (red) in the CA1 from a WT female; sections were DAPI-counterstained (blue) for neuronal nuclei (scale bar: 50 μ m). The higher magnification image (scale bar, 5 μ m) from the stratum pyramidale shows a PV neuron (right; merge in orange) and an unidentified neuron (left), both expressing Ambra1. The DAPI signal is omitted for clarity and neuron borders are indicated by a dash line ($n=3$ mice; three sections per animal). Image collected and cropped by CiteAb from the following publication (link.springer.com/article/10.1007/s12035-018-0911-5), licensed under a CC-BY license.



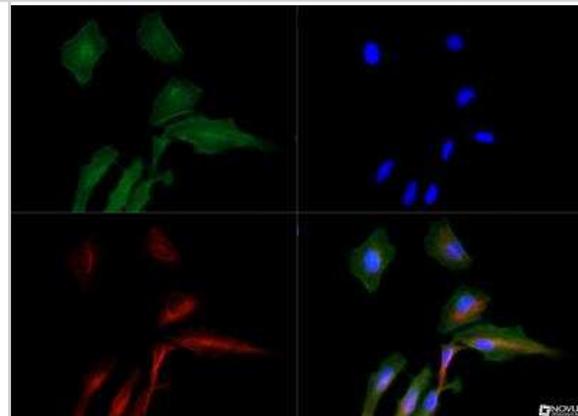
Western Blot: AMBRA1 Antibody [NBP1-07124] - WB analysis of AMBRA1 in mouse liver cell lysate.



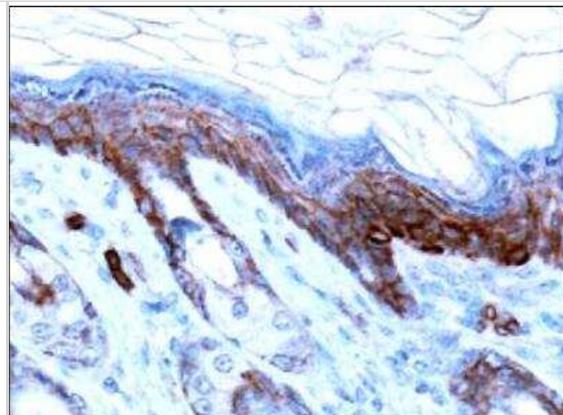
Immunohistochemistry: AMBRA1 Antibody [NBP1-07124] - IHC analysis of AMBRA1 in mouse brain using DAB with hematoxylin counterstain.



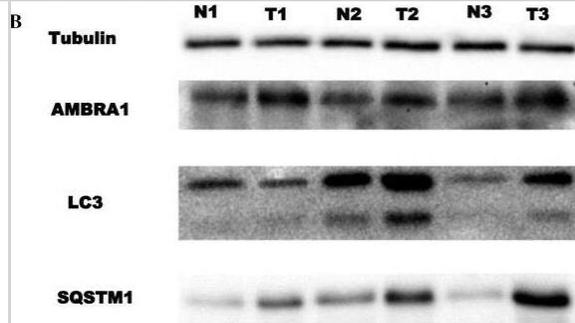
Immunocytochemistry/Immunofluorescence: AMBRA1 Antibody [NBP1-07124] - AMBRA1 antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



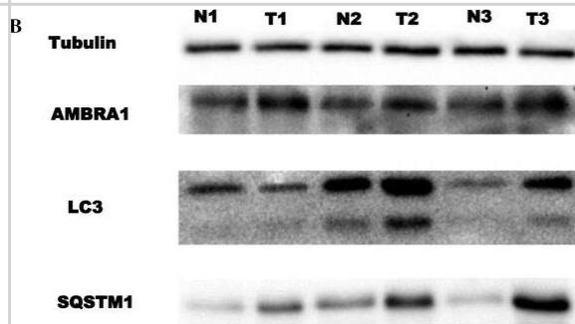
Immunohistochemistry: AMBRA1 Antibody [NBP1-07124] - IHC analysis of AMBRA1 in mouse epidermis using DAB with hematoxylin counterstain.



The protein levels of AMBRA1, LC3 and SQSTM1 were analyzed by western blot from fresh tissue samples. Tubulin was also detected as the control of sample loading. Representative western blots were shown. N normal tissue, T PCa tissue.



Western Blot: AMBRA1 Antibody - BSA Free [NBP1-07124] - a Immunohistochemical results showing the prevalence of AMBRA1 (left) & SQSTM1 (right) based on a semi-quantitative total score. Frequency distribution of the protein is reported according to the Gleason grade classification (GL6–GL8). A trend of AMBRA1 high score values correlation with the higher grade of Gleason score is visible. As opposite the levels of SQSTM1 positivity appeared higher in tumors with a lower Gleason score (Gleason 6). b The protein levels of AMBRA1, LC3 & SQSTM1 were analyzed by western blot from fresh tissue samples. Tubulin was also detected as the control of sample loading. Representative western blots were shown. N normal tissue, T PCa tissue. c mRNA expression of AMBRA1 & SQSTM1 clearly shows that a significant upregulation of these genes occurs in PCa in comparison with BPH. * $P < 0.01$; ° $P < 0.05$ Image collected & cropped by CiteAb from the following publication (<http://link.springer.com/10.1007/s10495-015-1176-3>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Antonioli M, Pagni B, Vescovo T et al. HPV sensitizes OPSCC cells to cisplatin-induced apoptosis by inhibiting autophagy through E7-mediated degradation of AMBRA1 Autophagy 2020-11-23 [PMID: 33172332] (Western Blot)

Ramirez-Pardo I, Villarejo-Zori B, Jimenez-Loygorri JI et al. Ambra1 haploinsufficiency in CD1 mice results in metabolic alterations and exacerbates age-associated retinal degeneration Autophagy 2022-07-24 [PMID: 35875981] (ICC/IF, Mouse)

Details:

Dilutions: 1:200

Li L, Zuo X, Liu D Et al. Plasma exosomal RNAs has potential as both clinical biomarkers and therapeutic targets of dermatomyositis Rheumatology (Oxford, England) 2021-10-26 [PMID: 34698812] (WB, IM)

Nobili A, Krashia P, Cordella A et al. Ambra1 Shapes Hippocampal Inhibition/Excitation Balance: Role in Neurodevelopmental Disorders. Mol. Neurobiol. 2018-02-27 [PMID: 29488136] (ICC/IF, Mouse)

Ugun-Klusek A, Tatham MH, Elkharaz J et al. Continued 26S proteasome dysfunction in mouse brain cortical neurons impairs autophagy and the Keap1-Nrf2 oxidative defence pathway Cell Death Dis 2017-01-05 [PMID: 28055010] (IF/IHC, Mouse)

Falasca L, Torino F, Marconi M et al. AMBRA1 and SQSTM1 expression pattern in prostate cancer. Apoptosis 2015-12-01 [PMID: 26423274] (WB)



Procedures

Serum protocol for AMBRA1 Antibody (NBP1-07124)

AMBRA1 Antibody:

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
 2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
 3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
 4. Rinse the blot.
 5. Block the membrane using standard blocking buffer for at least 1 hour.
 6. Wash the membrane in wash buffer three times for 10 minutes each.
 7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
 8. Wash the membrane in wash buffer three times for 10 minutes each.
 9. Apply the diluted 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 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 manufacturers instructions.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

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.

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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 NBP1-07124

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP1-07124B	AMBRA1 Antibody [Biotin]

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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