

Product Datasheet

ATP7b Antibody - BSA Free

NB100-360

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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NB100-360

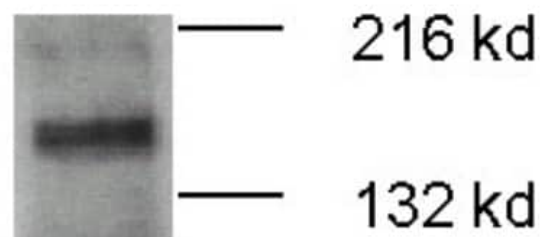
ATP7b Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	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	165 kDa
Product Description	
Host	Rabbit
Gene ID	540
Gene Symbol	ATP7B
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide made to an internal sequence near the N-terminus of human ATP7b. [UniProt# P35670]
Product Application Details	
Applications	Western Blot, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence 2 - 10 ug/mL, Immunoprecipitation, Immunohistochemistry-Paraffin 1:100 - 1:500, Immunoblotting reported in scientific literature (PMID 29250175)
Application Notes	In WB this antibody recognizes a band at 165 kDa, representing ATP7b and also recognizes a faint non-specific band at ~195 kDa. In IHC-P, cytoplasmic and golgi staining was observed in mouse liver tissue (1:100 dilution) and human ovarian carcinoma cells (1:500 dilution). However, please note we also tested this antibody with IHC-P in mouse intestines and kidney and observed unexpected strong nuclear signal with lighter cytoplasmic staining. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. 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.

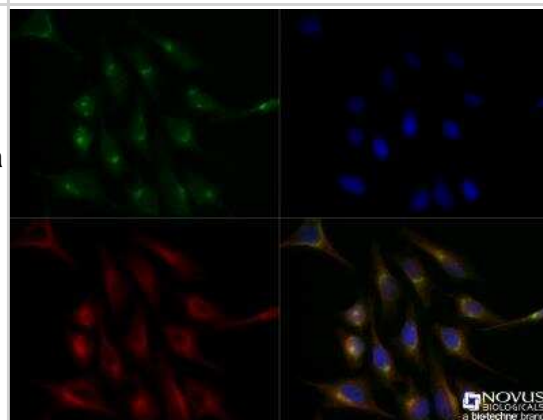


Images

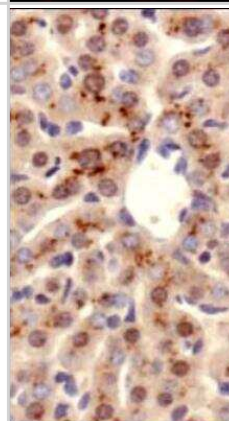
Western Blot: ATP7b Antibody [NB100-360] - ATP7b detected in 20 ug of mouse brain membrane fraction.



Immunocytochemistry/Immunofluorescence: ATP7b Antibody [NB100-360] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-ATP7b [NB100-360] at a 1:100 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at 1:500. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry: ATP7b Antibody [NB100-360] - Analysis of ATP7b in mouse liver using DAB with hematoxylin counterstain.



Publications

Tatsuo Ogawa, Kisho Ono, Shoji Ryumon, Hotaka Kawai, Tomoya Nakamura, Koki Umemori, Kunihiro Yoshida, Hideka Kanemoto, Kyoichi Obata, Norie Yoshioka, Tatsuo Okui, Kuniaki Okamoto, Hitoshi Nagatsuka, Soichiro Ibaragi Novel mechanism of cisplatin resistance in head and neck squamous cell carcinoma involving extracellular vesicles and a copper transporter system. Head & neck 2024-01-02 [PMID: 38164660]

Bonet-Aletá J, Pezacki A, Oi M et al. An Activity-Based Sensing Approach to Monitor Nanomaterial-Promoted Changes in Labile Metal Pools in Living Systems ChenRxiv 2023-06-15 (WB, Human)

Details:

Dilution 1:1000

Thepsuwan P, Bhattacharya A, Song Z et al. Hepatic SEL1L-HRD1 ER-associated degradation regulates systemic iron homeostasis via ceruloplasmin Proceedings of the National Academy of Sciences 2023-01-10 [PMID: 36595688] (Western Blot)

Shin VY, Liu MX, Siu JM et al. Inhibition of EP2 receptor suppresses tumor growth and chemoresistance of gastric cancer American journal of cancer research 2022-10-15 [PMID: 36381319] (WB, Human)

Bonet-Aleta J, Pezacki A, Oi M et al. Therapeutic Copper-based Nanoparticles Release Labile Copper(II) and Trigger Cellular Responses in Glutathione and NRF2 Redox Pathways and Metal Homeostasis ChemRxiv 2023-03-24 (WB)

Zhang S, Chen S, Li W et al. Rescue of ATP7B function in hepatocyte-like cells from Wilson's disease induced pluripotent stem cells using gene therapy or the chaperone drug curcumin Hum Mol Genet 4068-01-01 [PMID: 21593220] (FLOW, ICC/IF, Human)

Xia F, Fu Y, Xie H et al. Suppression of ATG4B by copper inhibits autophagy and involves in Mallory body formation Redox biology 2022-03-24 [PMID: 35349929] (WB, Human)

Yang D, Wang T, Liu J et al. Reverse regulation of hepatic ceruloplasmin production in rat model of myocardial ischemia J Trace Elem Med Biol 2020-11-16 [PMID: 33249375] (WB, Rat)

Details:

Western blot analysis performed on livers of Sprague-Dawley rats that underwent myocardial infarction.

Ryumon S, Okui T, Kunisada Y et al. Ammonium tetrathiomolybdate enhances the antitumor effect of cisplatin via the suppression of ATPase copper transporting beta in head and neck squamous cell carcinoma Oncol. Rep. 2019-10-10 [PMID: 31638244] (WB, IF/IHC, Human)

Moinuddin F M, Hirano Hirofumi, Shinsato Yoshinari et al. ATP7B expression in human glioblastoma is related to temozolomide resistance. Oncology Letters 2017-10-23 [PMID: 29250175] (IHC-P, IB, Human)

Han Y, Wang T, Liu H et al. The release and detection of copper ions from ultrasmall theranostic Cu₂-xSe nanoparticles Nanoscale 2019-06-20 [PMID: 31184674]

Agarwal T, Rustagi A, Das J, Maiti TK. PAMAM dendrimer grafted cellulose paper scaffolds as a novel in vitro 3D liver model for drug screening applications. Colloids Surf B Biointerfaces 2018-08-29 [PMID: 30179804] (Human)

More publications at <http://www.novusbio.com/NB100-360>



Procedures

Immunohistochemistry protocol for ATP7b Antibody (NB100-360)

Immunohistochemistry Procedure

Cell Preparation (At least 108 cells were used per block)

1. Harvesting cells:

A. Trypsinization

B. 15 minute centrifugation at 2,500 RPM

C. PBS rinse

D. 15 minute centrifugation at 2,500 RPM

2. Suspend cells in 10 ml of 10% formaldehyde in PBS, overnight @ RT.

3. Centrifuge cells at 2,500 RPM for 10 minutes.

4. Resuspend cells in 10 ml of 70% ethanol.

5. Centrifuge cells at 2,500 RPM and taken into 70% ethanol.

Cell Staining

1. Ribbon Thickness: 5 um

2. Deparaffination Agent: Xylin

3. Hydration: Ethanol in PBS

4. Antigen Retrieval: 10 minute microwave retrieval in citrate buffer; 20 minute cooling

5. Blocking:

A. endogeneous peroxidase: 0.3% H₂O₂ in PBS for 10 minutes

B. endogeneous protein: 1% BSA for 20 minutes

6. Primary antibody, polyclonal anti-ATP7b (NB 100-360): 1:500, overnight @ 4 degrees Celcius

7. Secondary antibody, anti-rabbit (HRP): (dilute per manufacturer recommendation), 30 minutes @ RT

8. Wash 3x 15 minutes

9. Chromogen: AEC

10. Counterstain: Mayers hematoxylin

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



Western Blot protocol for ATP7b Antibody (NB100-360)**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%.

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Immunocytochemistry/Immunofluorescence protocol for ATP7b Antibody (NB100-360)**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.

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Products Related to NB100-360

NBL1-07848	ATP7b Overexpression Lysate
NB100-360PEP	ATP7b 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

Limitations

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