

# Product Datasheet

## ATP7b Antibody - BSA Free NB100-361

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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**NB100-361**

ATP7b Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	165 kDa

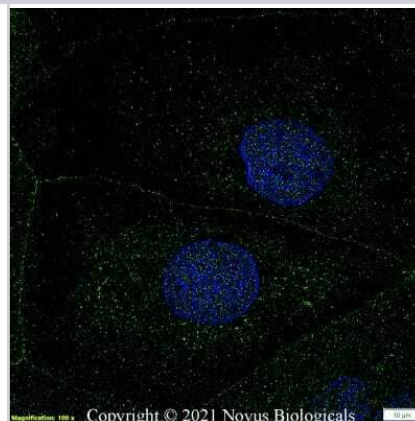
Product Description	
Description	Novus Biologicals Rabbit ATP7b Antibody - BSA Free (NB100-361) is a polyclonal antibody validated for use in WB and ICC/IF. Anti-ATP7b Antibody: Cited in 3 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	540
Gene Symbol	ATP7B
Species	Human, Rat, Mouse (Negative)
Reactivity Notes	Does not appear to react in mouse species.
Immunogen	A synthetic peptide made to an internal sequence near the C-terminus of human ATP7b. [UniProt# P35670]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 1:1000, Immunocytochemistry/ Immunofluorescence 1:10-1:2000
Application Notes	This ATP7b antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot. By WB, this antibody recognizes a band at 165 kDa, representing ATP7b. This antibody also recognizes a band at ~220 kDa. This antibody may work on endogenous protein in immunohistochemistry, but it has only been tested on transfected ovarian carcinoma cells at a 1:500 dilution. However, NB100-360 may demonstrate less background for this assay.

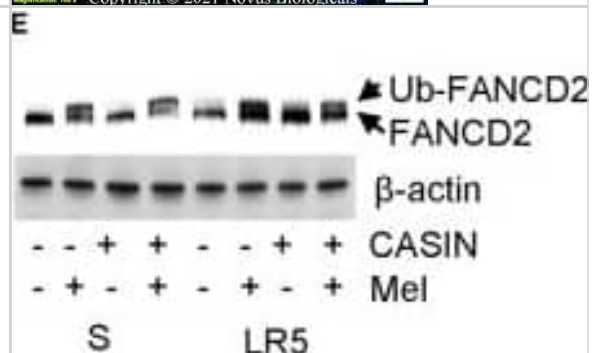


## Images

Immunocytochemistry/Immunofluorescence: ATP7b Antibody [NB100-361] - Caco-2 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 anti-ATP7b Antibody NB100-361 at 2 ug/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 100X objective and digitally deconvolved.



Western Blot: ATP7b Antibody [NB100-361] - Effects & mechanism of action of CASIN on melphalan-resistant MM cells. (A) CASIN reduces Cdc42 activity (Cdc42-GTP) but not Rac1 activity (Rac1-GTP). Melphalan-sensitive MM cells (S) were treated with Vehicle or CASIN (5  $\mu$ M) for 8 h. Cdc42 & Rac1 activities were measured using pull-down assay. (B) CASIN (5  $\mu$ M) inhibits proliferation of both S & melphalan-resistant (LR5) MM cells. \*\*P < 0.01 vs. S-Vehicle; ###P < 0.01 vs. LR5-Vehicle. (C) CASIN (5  $\mu$ M) preferentially causes apoptosis of melphalan-resistant MM cells. S & LR5 cells were treated with or without CASIN or melphalan (Mel, 25  $\mu$ M) or both for 2 days. Annexin V+ cells were analyzed using flow cytometry. \*\*P < 0.01. (D–F) Mechanism of action of CASIN on melphalan-resistant MM cells. (D) Cdc42 activity & expression are increased in LR5 cells compared to that in S cells. Cdc42 activity was measured using pull-down assay.  $\beta$ -Actin was used as loading control. Vertical lines indicate the gel lanes being switched in position from the original blots. (E) CASIN (5  $\mu$ M) decreases melphalan-induced FANCD2 mono-ubiquitination (Ub-FANCD2) in LR5 but not in S cells. S & LR5 cells were treated with or without CASIN, Mel (25  $\mu$ M), or both for 16 h. FANCD2 was detected using western blotting.  $\beta$ -Actin was used as loading control. (F) CASIN sensitizes melphalan-resistant but not -sensitive MM cells to melphalan-induced DNA damage.  $\gamma$  H2A histone family member X-positive ( $\gamma$ H2AX+) cells were detected using flow cytometry. \*\*P < 0.01. Error bars represent means  $\pm$  SD of triplicates & data are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31632904>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Nguyen P, Chakrabarti J, Li Y et al Rational Targeting of Cdc42 Overcomes Drug Resistance of Multiple Myeloma. *Frontiers in Oncology* 2019-10-01 [PMID: 31632904] (WB, Human)

Yoshizawa K, Nozaki S, Kitahara H et al. Copper efflux transporter (ATP7B) contributes to the acquisition of cisplatin-resistance in human oral squamous cell lines. *Oncol Rep.* 2007-10-01 [PMID: 17786364] (WB, Human)

Guo, Y et al. NH2-terminal signals in ATP7B Cu-ATPase mediate its Cu-dependent anterograde traffic in polarized hepatic cells. *Am J Physiol Gastrointest Liver Physiol*;289(5):G904-16. 2005-11-01 [PMID: 15994426] (WB, Rat, Human)

## Procedures

### Immunohistochemistry protocol for ATP7b Antibody (NB100-361)

ATP7b Antibody:

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  $\mu$ m

2. Deparaffination Agent: Xylin

3. Hydration: Ethanol in PBS

4. Blocking:

A. endogeneous peroxidase: 0.3% H<sub>2</sub>O<sub>2</sub> in PBS for 10 minutes

B. endogeneous protein: 1% BSA for 20 minutes

6. Primary antibody, polyclonal anti-ATP7b (NB 100-361): 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





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### **Products Related to NB100-361**

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NBL1-07848	ATP7b Overexpression Lysate
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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