

# Product Datasheet

## ATP13A2 Antibody - BSA Free NB110-41486

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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### Publications: 3

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**NB110-41486**

ATP13A2 Antibody - BSA Free

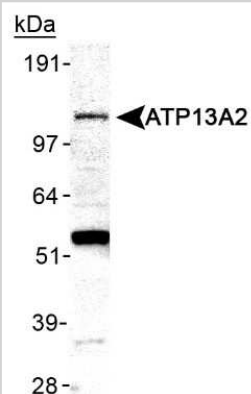
Product Information	
Unit Size	0.1 ml
Concentration	0.75 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Glycine and 0.15M NaCl

Product Description	
Description	Novus Biologicals Rabbit ATP13A2 Antibody - BSA Free (NB110-41486) is a polyclonal antibody validated for use in IHC, WB and Simple Western. Anti-ATP13A2 Antibody: Cited in 3 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	23400
Gene Symbol	ATP13A2
Species	Human, Mouse
Immunogen	A synthetic peptide made to an internal region of the human ATP13A2 protein (within residues 200-300). [Swiss-Prot# Q9NQ11]

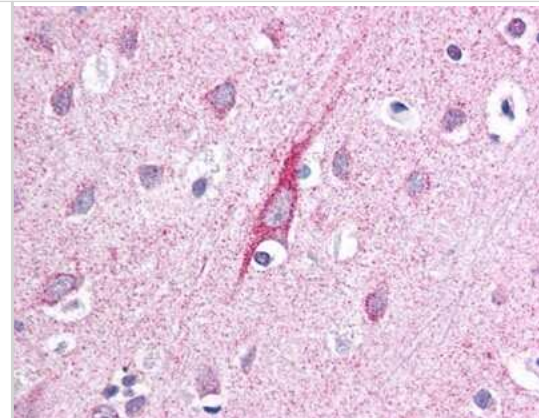
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry
Recommended Dilutions	Western Blot 2ug/ ml, Simple Western 1:100, Immunohistochemistry 10 ug/ml
Application Notes	This ATP13A2 antibody is useful for Immunohistochemistry and Western Blot.  In Simple Western 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 Human Brain lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100. Separated by Size-Wes, Sally Sue/Peggy Sue.

**Images**

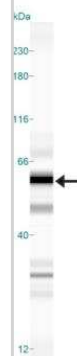
Western Blot: ATP13A2 Antibody [NB110-41486] - Detection of ATP13A2 in mouse brain membrane lysate.



Immunohistochemistry: ATP13A2 Antibody [NB110-41486] - Staining of neurons and neuropils using a concentration of 10ug/ml. Human brain, 40X.

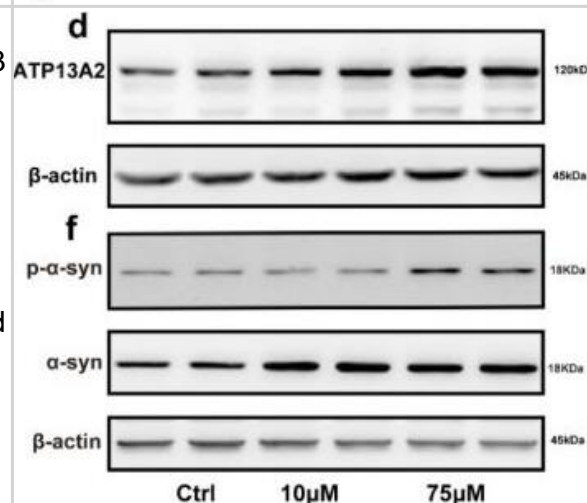


Simple Western: ATP13A2 Antibody [NB110-41486] - Simple Western lane view shows a specific band for ATP13A2 in 0.5 mg/ml of Human Brain lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

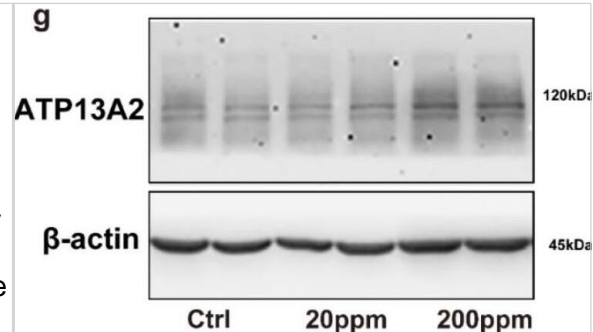


Effects of Zn<sup>2+</sup> on ATP13A2,  $\alpha$ -synuclein, and autophagy–lysosome pathway in HEK293  $\alpha$ -synuclein-DsRed cells. (a) Cell viability of HEK293  $\alpha$ -synuclein-DsRed cells incubated with different concentrations of Zn<sup>2+</sup> for 12 and 24 h. All values are presented as the mean  $\pm$  standard error of the mean (SEM) of at least three independent experiments. (b) TSM staining was used to verify the zinc fluorescence intensity in HEK293  $\alpha$ -synuclein-DsRed cells with a medium containing 10 or 75  $\mu$ M ZnSO<sub>4</sub>. Bar = 25  $\mu$ m. (c) HEK293  $\alpha$ -synuclein-DsRed cells were untreated or treated with 75  $\mu$ M ZnSO<sub>4</sub> for 12 h. Cells were observed using confocal microscopy after staining with ATP13A2 and DAPI. The spontaneous red fluorescence of HEK293  $\alpha$ -synuclein-DsRed cells represented the expression levels of  $\alpha$ -synuclein. Scale bar = 25  $\mu$ m. (d–m) HEK293  $\alpha$ -synuclein-DsRed cells were treated with 10 or 75  $\mu$ M ZnSO<sub>4</sub> for 12 h. Immunoblot images ((d,f), left panels) and quantification ((e,g,h), right panels) show the expression of ATP13A2 (d), p- $\alpha$ -synuclein and  $\alpha$ -synuclein (f) in the HEK293  $\alpha$ -synuclein-DsRed cells.

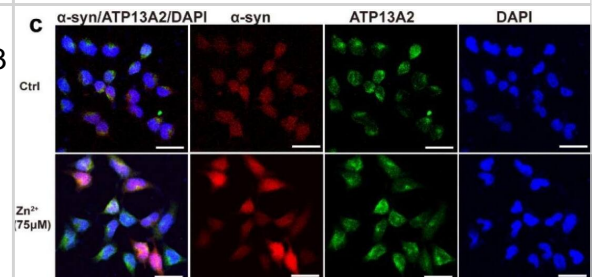
Autophagy–lysosome pathway ((i), representative pictures; (j–m) quantification): the expression levels of LAMP-1 (i,j), LAMP-2a (i,k), P62 (i,l), LC3 I and LC3 II ((i,m), quantification of ratio of LC3II to LC3I) were determined using Western blot analysis with  $\beta$ -actin as an internal control. All data from at least three independent experiments are represented as the means  $\pm$  SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$  with respect to the control group. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35887392>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Zn<sup>2+</sup> treatment reduces spatial exploration behavior and increases the expression levels of ATP13A2 protein in the brains of mice. The nine-month-old  $\alpha$ -synuclein-GFP mouse models were treated with different doses of ZnSO<sub>4</sub> (20 or 200 ppm) for three months. (a) Total distance traveled in open field exploration in 5 min. (b) Movement distance in zone center showing 5 min of open field exploration by the mice. (c) Time spent in the center zone showing 5 min of open field exploration by the mice. (d) Five minutes movement tracks of open field exploration by the mice. ((a–d), n = 10 mice per group) (e) Zinc levels in the brain tissue were determined using ICP-MS ((e), n = 6 mice per group), and expression of the ATP13A2 (g), representative pictures; (f), quantification; n = 6 mice per group) was analyzed using immunoblotting. (h) ATP13A2 positive reaction was detected in substantia nigra pars compacta cells via immunohistochemistry using ATP13A2 antibody (scale bar = 50  $\mu$ m) ((h), n = 6 mice per group). Data are represented as the means  $\pm$  SEM. The maximum and minimum values were removed from each group. Compared with the control group \* p < 0.05, \*\* p < 0.01. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35887392>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Effects of Zn<sup>2+</sup> on ATP13A2,  $\alpha$ -synuclein, and autophagy–lysosome pathway in HEK293  $\alpha$ -synuclein-DsRed cells. (a) Cell viability of HEK293  $\alpha$ -synuclein-DsRed cells incubated with different concentrations of Zn<sup>2+</sup> for 12 and 24 h. All values are presented as the mean  $\pm$  standard error of the mean (SEM) of at least three independent experiments. (b) TSSQ staining was used to verify the zinc fluorescence intensity in HEK293  $\alpha$ -synuclein-DsRed cells with a medium containing 10 or 75  $\mu$ M ZnSO<sub>4</sub>. Bar = 25  $\mu$ m. (c) HEK293  $\alpha$ -synuclein-DsRed cells were untreated or treated with 75  $\mu$ M ZnSO<sub>4</sub> for 12 h. Cells were observed using confocal microscopy after staining with ATP13A2 and DAPI. The spontaneous red fluorescence of HEK293  $\alpha$ -synuclein-DsRed cells represented the expression levels of  $\alpha$ -synuclein. Scale bar = 25  $\mu$ m. (d–m) HEK293  $\alpha$ -synuclein-DsRed cells were treated with 10 or 75  $\mu$ M ZnSO<sub>4</sub> for 12 h. Immunoblot images ((d,f), left panels) and quantification ((e,g,h), right panels) show the expression of ATP13A2 (d), p- $\alpha$ -synuclein and  $\alpha$ -synuclein (f) in the HEK293  $\alpha$ -synuclein-DsRed cells.



Autophagy–lysosome pathway ((i), representative pictures; (j–m) quantification): the expression levels of LAMP-1 (i,j), LAMP-2a (i,k), P62 (i,l), LC3 I and LC3 II ((i,m), quantification of ratio of LC3II to LC3I) were determined using Western blot analysis with  $\beta$ -actin as an internal control. All data from at least three independent experiments are represented as the means  $\pm$  SEM. \* p < 0.05, \*\* p < 0.01 with respect to the control group. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35887392>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

## Publications

Gao H, Sun H, Yan N et al. ATP13A2 Declines Zinc-Induced Accumulation of  $\alpha$ -Synuclein in a Parkinson's Disease Model International Journal of Molecular Sciences 2022-07-21 [PMID: 35887392] (Immunohistochemistry, Western Blot, Human)

Henry AG, Aghamohammadzadeh S, Samaroo H et al. Pathogenic LRRK2 mutations, through increased kinase activity, produce enlarged lysosomes with reduced degradative capacity and increase ATP13A2 expression. Hum. Mol. Genet. 2015-08-06 [PMID: 26251043] (WB, Human)

### Details:

ATP13A2 antibody used for WB on human post-mortem samples from prefrontal cortex of control, LRRK2 G2019S carriers and idiopathic PD patients (Figure 7).

Dehay B, Ramirez A, Martinez-Vicente M, Perier C, Canron MH, Doudnikoff E, Vital A, Vila M, Klein C, Bezdard E. Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. Proc Natl Acad Sci U S A. 2012-05-30 [PMID: 22647602] (IF/IHC, WB, Human)



## Procedures

### Western Blot protocol for ATP13A2 Antibody (NB110-41486)

ATP13A2 Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 32 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-ATP13A2 primary antibody (NB110-41486) 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's 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.

IHC-FFPE sections

I. Deparaffinization:

- A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
- B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

- A. Place slides in peroxidase quenching solution: 15-30 minutes.

To Prepare 200 ml of Quenching Solution:

Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.

Use within 4 hours of preparation

- B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

- A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
- B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
- C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
- D. Slowly add distilled water to further cool for 5 minutes.
- E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

- A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap-Pen).
- B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
- C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
- D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
- E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
- G. Wash slides with Wash Solution: 3 changes for 5 minutes each.



- H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
- I. Wash slides with Wash Solution: 3 changes for 5 minutes each. Wash slides with Wash Solution: 3 changes for 5 minutes each
- J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
- K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
- L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
- M. Rinse slides in distilled water.
- N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
- O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
- P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
- S. Lay slides on a flat surface to dry prior to viewing under microscope.

**NOTES:**

- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
- All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).





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### **Products Related to NB110-41486**

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NB110-41486PEP	ATP13A2 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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