

Product Datasheet

ATG5 Antibody - BSA Free NB110-53818

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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NB110-53818

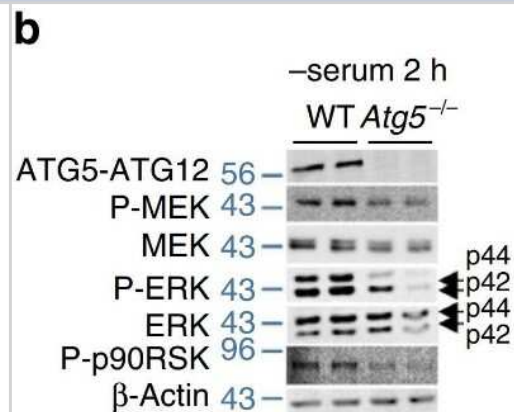
ATG5 Antibody - BSA Free

| Product Information | |
|------------------------------------|--|
| Unit Size | 0.1 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 | 0.02% Sodium Azide |
| Isotype | IgG |
| Purity | Immunogen affinity purified |
| Buffer | PBS |
| Target Molecular Weight | 32 kDa |
| Product Description | |
| Host | Rabbit |
| Gene ID | 9474 |
| Gene Symbol | ATG5 |
| Species | Human, Mouse, Rat, Porcine, Alligator, Bovine, Drosophila, Fish, Guinea Pig, Primate, Xenopus, Zebrafish |
| Reactivity Notes | Drosophila reactivity reported in scientific literature (PMID:33221768) Fish reactivity reported in scientific literature (PMID: 26183773). Guinea Pig reactivity reported in scientific literature (PMID: 30766882). Use in Alligator reported in scientific literature (PMID:32061056). |
| Specificity/Sensitivity | This is selective for the full-length and calpain cleaved isoform proteins. The short isoform is missing amino acids 1-79. The calpain cleaved form of ATG5 is missing amino acids 195-275. |
| Immunogen | This ATG5 Antibody was made to a synthetic peptide of an N-terminal region of the human ATG5 protein (within residues 1-50) [Swiss-Prot Q9H1Y0]. |
| Product Application Details | |
| Applications | Western Blot, Simple Western, ELISA, Electron Microscopy, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Radioimmunoassay, Knockdown Validated, Knockout Validated |
| Recommended Dilutions | Western Blot 1:500, Simple Western 1:50, Flow Cytometry, ELISA, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 1:250, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:400, Immunoblotting, Radioimmunoassay, Proximity Ligation Assay, Electron Microscopy, Knockout Validated, Knockdown Validated |



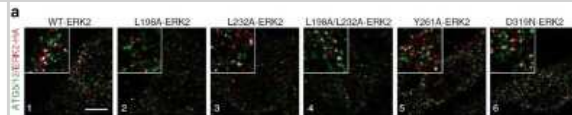
Images

Western Blot: ATG5 Antibody - BSA Free [NB110-53818] - ERK phosphorylation in *Atg5*^{-/-} cells depends on nutrient availability. Serum-deprived *Atg5*^{-/-} MEFs display decreased ERK and p90RSK phosphorylation. Immunoblots for the indicated proteins in total lysates from 2 h serum-deprived WT and *Atg5*^{-/-} MEFs. The bars represent mean \pm s.e.m. **P* < 0.05, ****P* < 0.001 compared with Con; Student's t-test, *n* = 3. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/ncomms3799>), licensed under a CC-BY license.

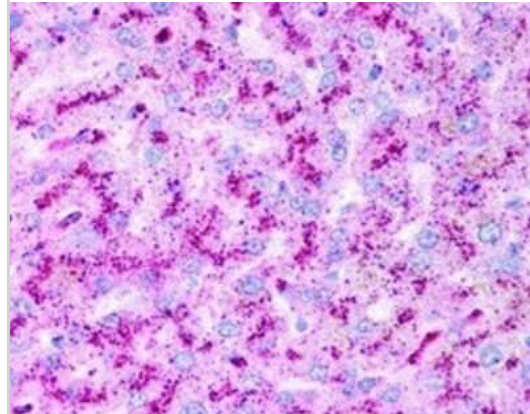


Immunocytochemistry/Immunofluorescence: ATG5 Antibody - BSA Free [NB110-53818] - ERK2 utilizes kinase-docking domains to interact with ATG5-ATG12 and LC3-II. (a) Mutations in FRS on ERK2 decrease colocalization of ERK2 with ATG5-ATG12, LC3 and WIPI1.

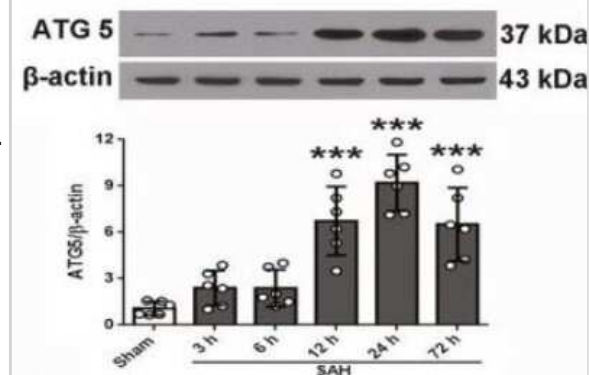
Immunofluorescence (IF) showing colocalization (depicted as white pixels by colocalization finder application) of WT-ERK2-HA, FRS ERK2 mutants (L198A-, L232A-, L198A/L232A-, Y261A-ERK2-HA) or common docking (CD) mutant (D319N-ERK2-HA) with ATG5-ATG12 (panels 1-6), LC3 (panels 7-12) or WIPI1 (panels 13-18) in EGF-treated NIH/3T3 cells. ERK2 is stained in red, and autophagy proteins are stained in green. Scale bar, 10 μ m. The bars represent mean \pm s.e.m. ***P* < 0.01, *****P* < 0.0001 compared with WT-ERK2-transfected cells; Student's t-test, 50 cells analysed from *n* = 2. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/ncomms3799>), licensed under a CC-BY license.



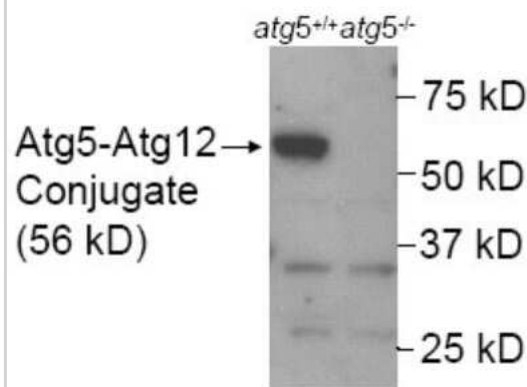
Immunohistochemistry: ATG5 Antibody - BSA Free [NB110-53818] - Immunohistochemistry analysis of human liver hepatocytes at 2.5 μ g/mL. 40X magnification.



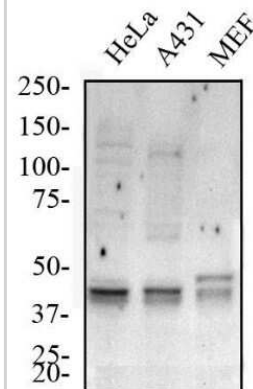
Western Blot: ATG5 Antibody - BSA Free [NB110-53818] - Representative Western blot images and quantitative analyses of ATG5 from the left hemisphere of rat brains at different time points after SAH. Sample size is 36, *n* = 6 per group. Data were presented as mean \pm SD. *F* = 22.14 for ATG5. **P* < .05, ** *P* < .01, ****P* < .001 vs Sham group. SAH, subarachnoid hemorrhage; Vehicle, phosphate-buffered saline; rOPN, recombinant OPN. NS, not significant. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31436915/>) licensed under a CC-BY license.



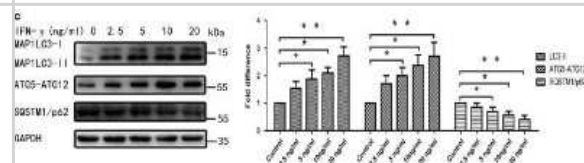
Western Blot: ATG5 Antibody - BSA Free [NB110-53818] - Western blot analysis in mouse wildtype ES cell lysate (Lane 1) using NB110-53818. Lane 2 is a mouse ATG5 KO ES cell lysate (negative control). Atg5^{-/-} ES cells from Dr. Noboru Mizushima [Mizushima, N. et al. J. Cell Biol. 152 (2001)] Photo courtesy of Dr. Beth Levine, UT Southwestern Medical Center.



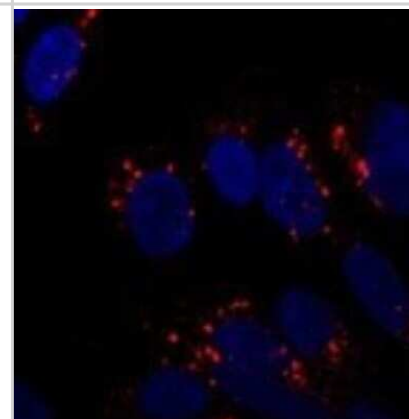
Western Blot: ATG5 Antibody - BSA Free [NB110-53818] - Western blot analysis of total protein from Human HeLa and A431 and Mouse MEF cells. Lysates were separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/mL anti-ATG5 in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



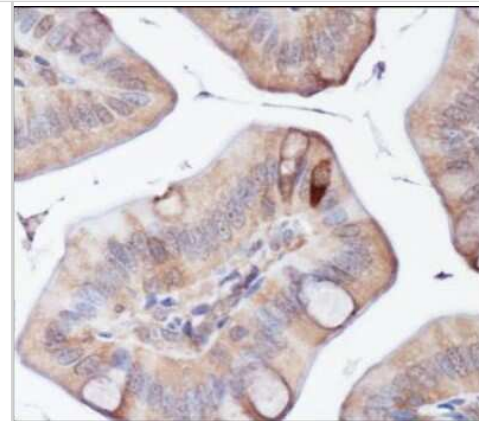
Western Blot: ATG5 Antibody - BSA Free [NB110-53818] - IFN-gamma increased rates of autophagy in primary BMECs in vitro. BMECs were treated with IFN-gamma at 2.5, 5, 10 or 20 ng/mL. At the end of the treatment, levels of MAP1LC3, ATG12-ATG5, SQSTM1/p62 and GAPDH were analyzed using Western blot analysis with specific antibodies as described in the Materials and Methods section. The data represent the mean \pm S.E.M. of three independent experiments. Error bars are \pm S.E.M. One-way ANOVA; *P<0.05; **P<0.01. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/cddiscovery201565>) licensed under a CC-BY license.



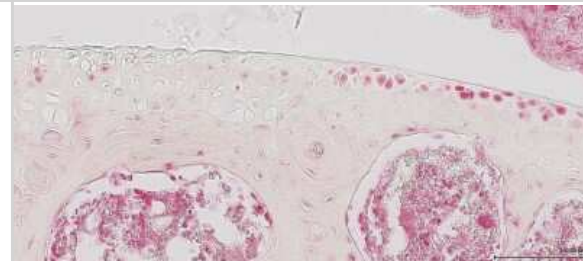
Immunocytochemistry/Immunofluorescence: ATG5 Antibody - BSA Free [NB110-53818] - ICC/IF analysis of SY5Y cells at 1:250. Incubated overnight at 4 degrees. Photo courtesy of an anonymous collaborator.



Immunohistochemistry: ATG5 Antibody - BSA Free [NB110-53818] - Immunohistochemistry analysis of mouse intestine using DAB with hematoxylin counterstain.



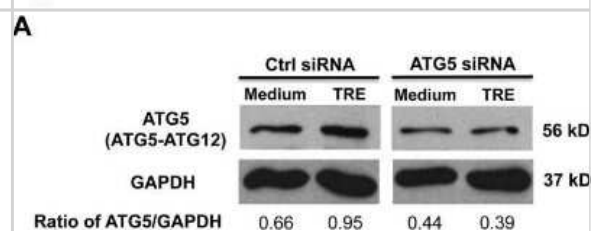
Immunohistochemistry-Paraffin: ATG5 Antibody - BSA Free [NB110-53818] - Immunohistochemistry analysis of mouse knee. IHC-P image submitted by a verified customer review.



Simple Western: ATG5 Antibody - BSA Free [NB110-53818] - Simple Western lane view shows a specific band for ATG5 in 0.5 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

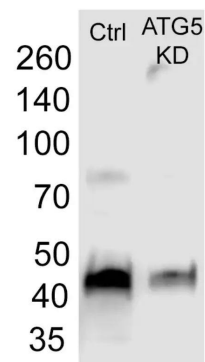


Knockdown Validated: ATG5 Antibody - BSA Free [NB110-53818] - Knockdown of autophagy-related gene 5 (ATG5) inhibits trehalose-induced autophagy in normal human primary airway epithelial cells. Normal human tracheobronchial epithelial cells were transfected with Naito1 chimera RNAi (control siRNA) or ATG5 chimera siRNA (ATG5 siRNA). Twenty-four hours after siRNA transfection, cells were treated with medium or trehalose (TRE, 100 mM) for 48 h. ATG5 protein as examined by Western blot analysis with GAPDH protein used as loading control. The representative Western blot picture was shown from 2 independent experiments with each being performed in triplicate wells.

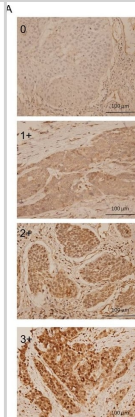


Western Blot: ATG5 Antibody - BSA Free [NB110-53818] - Western Blot of the lysate of DU145 cells - control and ATG5 KD. Image from verified customer review.

MW, KDa



(A) ATG5 expression in ESCC tissue was analyzed by IHC and scored as 0, 1+, 2+, and 3+. (B) Expression level of ATG5 in adjacent non-cancerous (normal) and early-stage ESCC tissues by IHC. (C-D) Kaplan–Meier estimates of OS (C) and PFS (D) by the expression levels of ATG5 (low and high) of adjacent normal tissue from early-stage ESCC patients. MST: median survival time. Low, expression score 0 or 1+; High, expression score 2+ or 3+.



Publications

Mukhopadhyay S, Praharaj PP, Naik PP et al. Identification of Annexin A2 as a key mTOR target to induce autophagy to autophagy dependent cell death in stress *Biochim Biophys Acta Mol Basis Dis* 2020-08-22 [PMID: 32841734]

Rebecca Z. Fan, Carolina Sportelli, Yanhao Lai, Said S. Salehe, Jennifer R. Pinnell, Harry J. Brown, Jason R. Richardson, Shouqing Luo, Kim Tieu A partial Drp1 knockout improves autophagy flux independent of mitochondrial function *Molecular Neurodegeneration* 2024-03-19 [PMID: 38504290]

Mathai BJ, Meijer AH, Simonsen A. Studying Autophagy in Zebrafish. *Cells* 2017-07-09 [PMID: 28698482]

Nah J, Pyo JO, Jung S et al. BECN1/Beclin 1 is recruited into lipid rafts by prion to activate autophagy in response to amyloid beta 42. *Autophagy* 2013-12-01 [PMID: 24145555]

S Mukhopadhy, J Encarnació, EY Lin, ASW Sohn, H Zhang, JD Mancias, AC Kimmelman Autophagy supports mitochondrial metabolism through the regulation of iron homeostasis in pancreatic cancer *Science Advances*, 2023-04-19;9(16):eadf9284. 2023-04-19 [PMID: 37075122]

Park SM, Lim JS, Ramakrishina S et al. Brain Somatic Mutations in MTOR Disrupt Neuronal Ciliogenesis, Leading to Focal Cortical Dyslamination *Neuron* 2018-06-09 [PMID: 29937275]

Shounak Baksi, Nihar R. Jana, Nitai Pada Bhattacharyya, Debashis Mukhopadhyay, Kah-Leong Lim Grb2 Is Regulated by Foxd3 and Has Roles in Preventing Accumulation and Aggregation of Mutant Huntingtin *PLoS ONE* 2013-10-08 [PMID: 24116161]

Sathyanarayan A, Mashek MT, Mashek DG. ATGL Promotes Autophagy/Lipophagy via SIRT1 to Control Hepatic Lipid Droplet Catabolism. *Cell Rep.* 2017-04-04 [PMID: 28380348]

Bi Y, Yang G, Guo Z et al. Chronic high salt intake induces cardiomyocyte autophagic vacuolization during left ventricular maladaptive remodeling in spontaneously hypertensive rats *Experimental and Therapeutic Medicine* 2023-02-16 [PMID: 36911373]

MOROKI T, MATSUO S, HATAKEYAMA H et al. Databases for technical aspects of immunohistochemistry-2021 update *Journal of Toxicologic Pathology* 2021-02-24 [PMID: 33976473]

Francesco P Zummo, Kirsty S Cullen, Minna Honkanen-Scott, James A M Shaw, Penny E Lovat, Catherine Arden Glucagon-Like Peptide 1 Protects Pancreatic β -Cells From Death by Increasing Autophagic Flux and Restoring Lysosomal Function. *Diabetes* 2017-08-18 [PMID: 28232493]

Park HJ, Jo DS, Choi H et al. Melasolv induces melanosome autophagy to inhibit pigmentation in B16F1 cells *PLoS one* 2020-09-17 [PMID: 32941497]

More publications at <http://www.novusbio.com/NB110-53818>



Procedures

Western Blot protocol for ATG5 Antibody (NB110-53818)

Protocol: Western Blot Protocol for Atg5 Antibody (NB110-53818)

Materials

- 1X PBS
- Sample buffer, 2X Laemmli buffer: 4% SDS, 5% 2-mercaptoethanol (BME), 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8
- 1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
- 1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol Adjust to pH 8.3
- TBS
- TBST, TBS and 0.1% Tween
- Blocking solution: TBST, 5% non-fat dry milk
- rabbit anti-Atg5 primary antibody (NB110-53818) in blocking buffer (1:500)

Methods

1. Grow cells (e.g. HeLa or Neuro2A) in vitro to semi-confluency (70-75%).
2. Rinse cells with ice-cold 1X PBS and lyse cells with sample buffer.
3. Sonicate and incubate cells for 5 minutes at 95°C.

Tip: Cells are lysed directly in sample buffer.

4. Load 10-40 µg/lane of sample on a 12% polyacrylamide gel (SDS-PAGE).
5. Transfer proteins to a PVDF membrane for 60 minutes at 100V.

Tip: For more information on Western Blotting, see our Western Blot handbook:

6. After transfer, rinse the membrane with dH₂O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.
7. Rinse the membrane in dH₂O to remove excess stain and mark the loaded lanes and molecular weight markers using a pencil.
8. Block the membrane using blocking buffer solution (5% BSA in TBST) for 1 hour at room temperature.
9. Rinse the membrane with TBST for 5 minutes.
10. Dilute anti-Atg5 primary antibody (NB110-53818) in blocking buffer (1:500) and incubate the membrane for 1 hour at room temperature.
11. Rinse the membrane with dH₂O.
12. Rinse the membrane with TBST, 3 times for 10 minutes each.
13. Incubate the membrane with diluted secondary antibody, according with product's specification, (e.g. anti-rabbit-IgG HRP-conjugated) in blocking buffer for 1 hour at room temperature.

Note: Tween-20 may 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.

14. Rinse the membrane with TBST, 3 times for 10 minutes each.
15. Apply the detection reagent of choice (e.g. BioFX Super Plus ECL) in accordance with the manufacturer's instructions.

Deparaffinization:

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

Quench Endogenous Peroxidase:

1. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200ml of Quenching Solution: Add 3ml of 30% Hydrogen Peroxide to 200ml of Methanol. Use within 4 hours of preparation.
2. Place slides in distilled water: 2 changes for 2 minutes each.

Retrieve Epitopes:

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

Immunostaining Procedure:

1. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap-Pen).
2. Flood slide with wash solution. Do not allow tissue sections to dry for the rest of the procedure.
3. Drain wash solution and apply 4 drops of blocking reagent to each slide and incubate for 15 minutes.
4. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200ul of primary antibody solution to each slide, and incubate for 1 hour.
5. Wash slides with wash solution: 3 changes for 5 minutes each.
6. Drain wash solution, apply 4 drops of secondary antibody to each slide and incubate for 1 hour.
7. Wash slides with wash solution: 3 changes for 5 minutes each.
8. Drain wash solution, apply 4 drops of DAB substrate to each slide and develop for 5-10 minutes. Check development with microscope.
9. Wash slides with wash solution: 3 changes for 5 minutes each.
10. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
11. Wash slides with wash solution: 2-3 changes for 2 minutes each.
12. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
13. Rinse slides in distilled water.
14. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
15. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
16. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
17. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
18. Apply 2-3 drops of non-aqueous mounting media to each slide.
19. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

- Use treated slides (e.g. HistoBond) to ensure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60 degrees C 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.
- 200ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections, less than 200ul 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 1-2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired, increase the time (up to 10 minutes).



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Products Related to NB110-53818

| | |
|----------------|---|
| NBL1-07799 | ATG5 Overexpression Lysate |
| NB110-53818PEP | ATG5 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

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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