Product Datasheet

ATG5 Antibody
NB110-53818SS

Unit Size: 0.025 ml

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

Reviews: 4 Publications: 73

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ATG5 Antibody

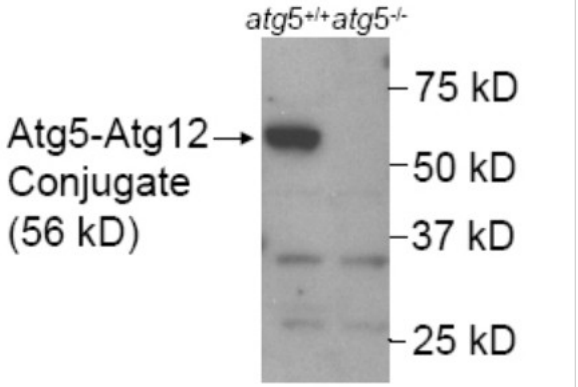
Product Information	
Unit Size	0.025 ml
Concentration	1.16 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol

Product Description	
Host	Rabbit
Gene ID	9474
Gene Symbol	ATG5
Species	Human, Mouse, Rat, Bovine, Primate, Porcine, Xenopus, Zebrafish
Species Reactivity	Human, mouse, rat, cow, pig, primate, Xenopus, and Zebrafish.
Specificity/Sensitivity	This is selective for the full-length and calpain cleaved isoform proteins. The short isoform is missing a.a. 1-79. The calpain cleaved form of ATG5 is missing a.a. 195-275.
Immunogen	A synthetic peptide made to an N-terminal region of the human ATG5 protein (within residues 1-50) [Swiss-Prot Q9H1Y0]

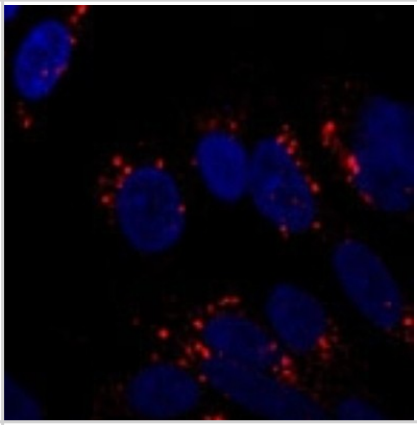
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:250, Immunohistochemistry 1:400, Immunohistochemistry-Paraffin 1:400, Western Blot 1:500, Immunoprecipitation, Simple Western 1:50
Application Notes	This ATG5 antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence and Immunohistochemistry-paraffin embedded sections. In Western Blot, a band is seen ~56 kDa representing the ATG5-ATG12 complex, the molecular weight of human ATG5 is ~33 kDa. In ICC/IF, cytoplasmic staining was observed in SY5Y cells. In IHC-P, staining was observed in the cytoplasm of human hepatocytes and mouse intestine tissues. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. Use in Immunoprecipitation reported in scientific literature (PMID 24705551)In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

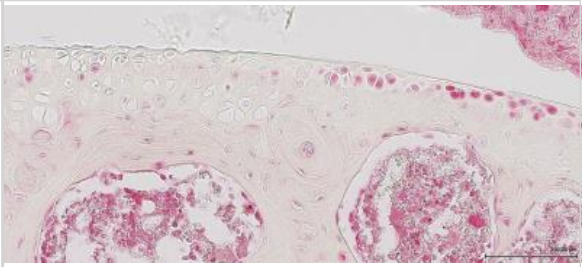
Western Blot: ATG5 Antibody [NB110-53818] - Detection of ATG5 in mouse wildtype ES cell lysate (Lane 1) using NB110-53818. Lane 2 is a mouse ATG5 KO ES cell lysate (negative control). Atg5-/- ES cells from Dr. Noboru Mizushima [Mizushima, N. et al. J. Cell Biol. 152 (2001)] Photo courtesy of Dr. Beth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



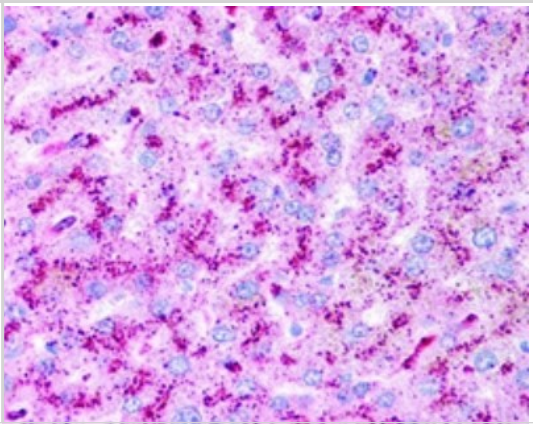
Immunocytochemistry/Immunofluorescence: ATG5 Antibody [NB110-53818] - Staining of SY5Y cells using NB110-53818 at 1:250. Incubated overnight at 4 degrees. Photo courtesy of an anonymous collaborator.

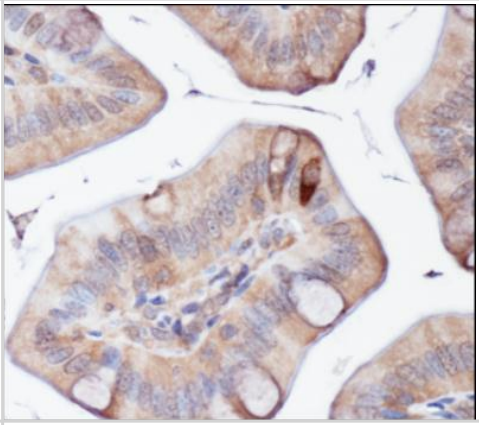


Immunohistochemistry-Paraffin: ATG5 Antibody [NB110-53818] - Staining of mouse knee using anti-ATG5 antibody. Image from verified customer review.



Immunohistochemistry: ATG5 Antibody [NB110-53818] - Staining of ATG5 in human liver hepatocytes using NB110-53818 at 2.5ug/ml. 40X magnification.



Immunohistochemistry: ATG5 Antibody [NB110-53818] - Analysis of ATG5 in mouse intestine using DAB with hematoxylin counterstain.	
Simple Western: ATG5 Antibody [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.	

Publications

Lin HH. In Vitro and in Vivo Atheroprotective Effects of Gossypetin against Endothelial Cell Injury by Induction of Autophagy Chem. Res. Toxicol. 2015 Jan 29 [PMID: 25622137] (WB, Human)

Malhotra R, Warne JP, Salas E et al. Loss of Atg12, but not Atg5, in pro-opiomelanocortin neurons exacerbates diet-induced obesity Autophagy. 2015 Jan 13 [PMID: 25585051] (WB)

Varga M, Fodor E, Vellai T. Autophagy in zebrafish. Methods. 2014 Dec 09 [PMID: 25498006] (WB, Zebrafish)

Weber K, Schilling JD. Distinct Lysosome Phenotypes Influence Inflammatory Function in Peritoneal and Bone Marrow-Derived Macrophages. International Journal of Inflammation. 2014 Dec 23 (WB, Mouse)

Details:
ATG5 antibody used for WB on the total cellular protein lysates of Peritoneal macrophage / pMACs or Bone marrow-derived macrophage / BMDMs isolated from C57BL/6 mice (Figure 4g)

Sun KT, Chen MY, Tu MG et al. MicroRNA-20a regulates autophagy related protein-ATG16L1 in hypoxia-induced osteoclast differentiation. Bone. 2014 Dec 05 [PMID: 25485521] (WB, Mouse)

Details:
ATG5 antibody used for WB on RAW264.7 cells subjected to RANKL and M-CSF mediated osteoclast differentiation as well as hypoxic stress (Fig. 1E)

Lin Hh, Lin Sm, Chung Y et al. Dynamic involvement of ATG5 in cellular stress responses. Cell Death Dis. 2014 Oct 24 [PMID: 25341032] (WB, IHC-P, Mouse)

Rozman S, Yousefi S, Oberson K et al. The generation of neutrophils in the bone marrow is controlled by autophagy. Cell Death Differ. 2014 Oct 17 [PMID: 25323583] (WB, Mouse)

Liao Cl, Chen Cm, Chang Yz et al. Pine (Pinus morrisonicola Hayata) needle Extracts Sensitize GBM8901 Human Glioblastoma Cells to Temozolomide by Downregulating Autophagy and O(6)-Methylguanine-DnA Methyltransferase Expression. J. Agric. Food Chem. 2014 Oct 29 [PMID: 25293350] (WB, Human)

Kao C, Chao A, Tsai Cl et al. Bortezomib enhances cancer cell death by blocking the autophagic flux through stimulating ERK phosphorylation Cell Death Dis et al. 2014 Nov 07 [PMID: 25375375] (WB, Human)

Details:
ATG5 antibody used for WB on ovarian cancer TOV112D cells treated or not with bortezomib/BTZ.

El-Hage N, Rodriguez M, Dever Sm et al. HIV-1 and morphine regulation of autophagy in microglia: Limited interactions in the context of HIV-1 infection and opioid abuse J. Virol. 2014 Oct 29 [PMID: 25355898] (WB, Human)

Details:
ATG5 antibody used for WB at 1:1000 dilution on lysates of primary human microglial cells that were treated or not with HIV-1SF162 macrophage (M)-tropic strain and/or Morphine for 1, 3 or 5 days (Figure 1B).

Granato M, Santarelli R, Farina A et al. EBV blocks the autophagic flux and appropriates the autophagic machinery to enhance viral replication. J Virol. 2014 Aug 20 [PMID: 25142602] (WB, Primate)

Montaigne D, Marechal X, Coisne A et al. Myocardial Contractile Dysfunction is Associated with Impaired Mitochondrial Function and Dynamics in Type 2 Diabetic but not in Obese Patients. Circulation. 2014 Jun 13 [PMID: 24928681] (WB, Human)

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

Procedures

Western Blot Protocol for ATG5 Antibody (NB110-53818)
Western blot protocol for detecting the Atg5-Atg12 conjugate

Preparation of Protein Samples
1. Wash cells with PBS once, then add 0.05% trypsin-EDTA for 1 minute at 37 degrees C.
2. Wash the collected cells with cold PBS on ice 3 times.
3. Add Tris-HCl lysis buffer (50 mM Tris-HCl; 150mM NaCl; 1mM EDTA; 100ug/ml PMSF; 1% Triton X-100; 1% protease inhibitor).
4. Rotate for 1 hour at 4 degrees C.
5. Collect the lysate by centrifuging 13,000rpm for 10 min at 4 degrees C.
6. Add Laemmli sample buffer (BIO-RAD, Cat. No. 161-0737) with Mercaptoethanol to the lysates and boil the lysates for 5 minutes at 100 degrees C.

Western Blot
1. Proteins were separated on a 12% SDS-PAGE gel. Approximately 10ug of protein was loaded per lane.
2. Blocking: 5% non-fat dry milk in PBS + 0.1% TWEEN-20 [PBS-T] for 1 hour at room temperature.
3. Incubate the membrane with anti-Atg5 antibody (NB 110-53818) in 5% non-fat dry milk in 1xPBS-T (1:500) at room temperature for 1 hour.
4. Wash the membrane 3 times with PBS-T, 10 min each wash.
5. Incubate the membrane with anti-rabbit secondary antibody in 5% non-fat dry milk in PBS-T at room temperature for 1 hour.
6. Wash the membrane 3 times with PBS-T, 10 minutes for each wash.
7. Add chemiluminescent substrate (PIERCE, Cat. No. 34077) for 5 minutes at room temperature.
8. Develop the film.

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).

Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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 guarantee, please visit www.novusbio.com/guarantee.