Product Datasheet

p62/SQSTM1 Antibody - BSA Free
NBP1-48320

Unit Size: 0.1 ml

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

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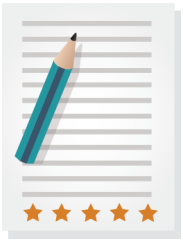
Updated 4/13/2025 v.20.1



NBP1-48320

p62/SQSTM1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 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

Product Description

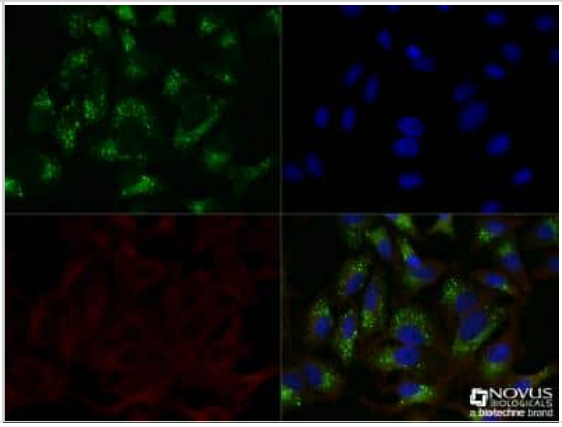
Host	Rabbit
Gene ID	8878
Gene Symbol	SQSTM1
Species	Human, Mouse, Rat, Chicken
Reactivity Notes	Use in Chicken reported in scientific literature (PMID:33360697) Immunogen sequence is 100% identical to several non-human primates/monkey species. Immunogen displays the following percentage of sequence identity for non-tested species: Chinese hamster (98%), Sheep (98%), Bovine (97%), Porcine (96%), Canine (91%), Daphnia magna, a cladoceran/crustaceans invertebrate (97%) and Duck and several other Birds (84%).
Immunogen	Partial recombinant protein made to the C terminal of the human p62/SQSTM1 protein (within residues 300-440). [Swiss-Prot Q13501]

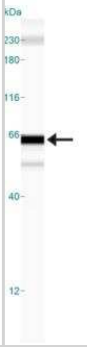
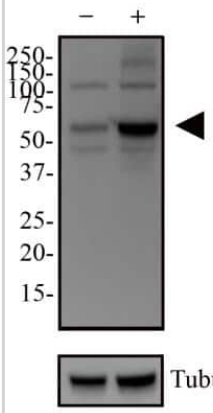
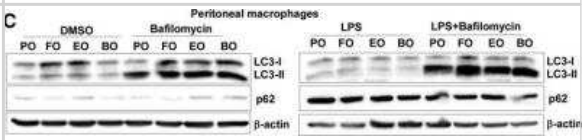
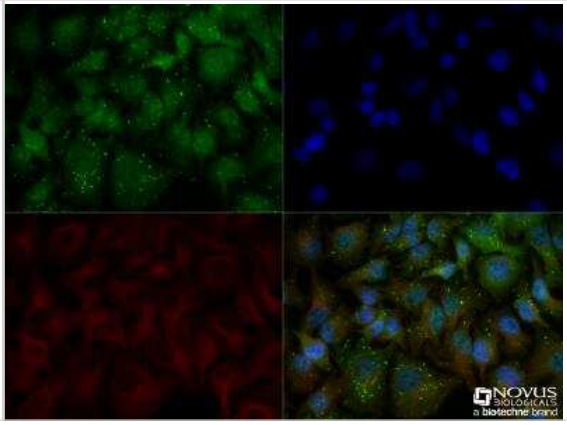
Product Application Details

Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunohistochemistry Free-Floating
Recommended Dilutions	Western Blot 1:4000, Simple Western 1:25, Flow Cytometry, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:25-1:200, Immunohistochemistry-Paraffin, Immunohistochemistry-Frozen, Immunohistochemistry Free-Floating reported in scientific literature (PMID 35389045)

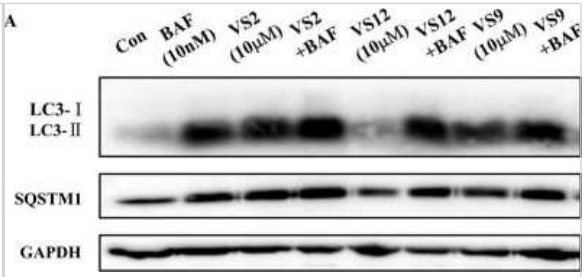
Images

Immunocytochemistry/Immunofluorescence: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - HeLa cells were treated overnight with 50uM CQ, then 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-p628/SQSTM1 at a 1:200 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 a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

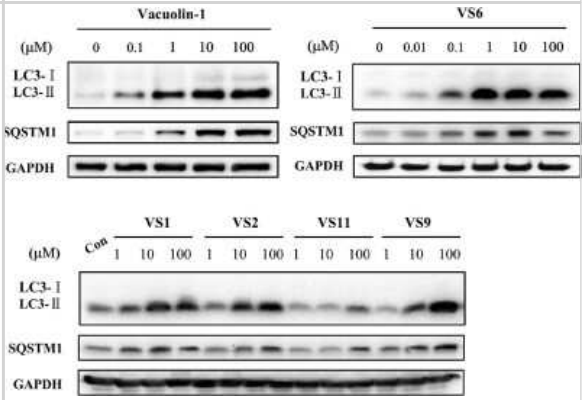


Simple Western: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - Lane view shows a specific band for p62/SQSTM1 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - Cultured HeLa cells were treated with or without 50 uM chloriquine for 24 hours as indicated. Cell lysates were prepared and separated on a 12% gel by SDS-PAGE. Protein was transferred to PVDF membrane and blocked in 5% non-fat milk. The membrane was then probed with 1 ug/ml anti-p62/SQSMT1 in 1% milk and dectected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the upregulation of p62 (arrowhead) in response to chloroquine treatment and the blockage of autophagy.	
Western Blot: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - Immunoblotting analysis of LC3 and p62/SQSTM1 expression in thioglycollate-elicited peritoneal macrophages. Macrophages were isolated from C57BL/6 mice fed diets for 12 weeks, treated with or without 100 ng/ml LPS for 18 h with or without 50 nM bafilomycin A1. Image collected and cropped by CiteAb from the following publication (https://linkinghub.elsevier.com/retrieve/pii/S0022227520339523), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - Untreated 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-p628/SQSTM1 at a 1:200 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 a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	

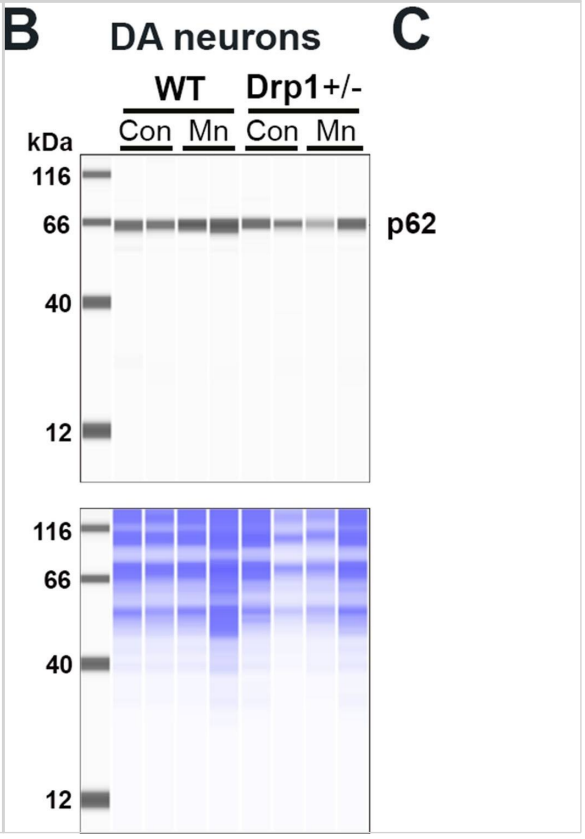
Western Blot: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - Vacuolin-1 analogues identified via virtual screening inhibited autophagic flux in HeLa cells. (A) Treatment of HeLa cells with vacuolin-1 analogues (10 M) and BAF (100 nM) failed to further increase the accumulation of both LC3B-II and SQSTM1 as compared to either drug alone. (B) Vacuolin-1 (10 M) or VS6 (10 M) induced the accumulation of yellow LC3B-II puncta in RFP-GFP-LC3B expressing HeLa cells. Scale bar = 10 m. Identification of Novel Vacuolin-1 Analogues as Autophagy Inhibitors by Virtual Drug Screening and Chemical Synthesis. *Molecules* (2017)



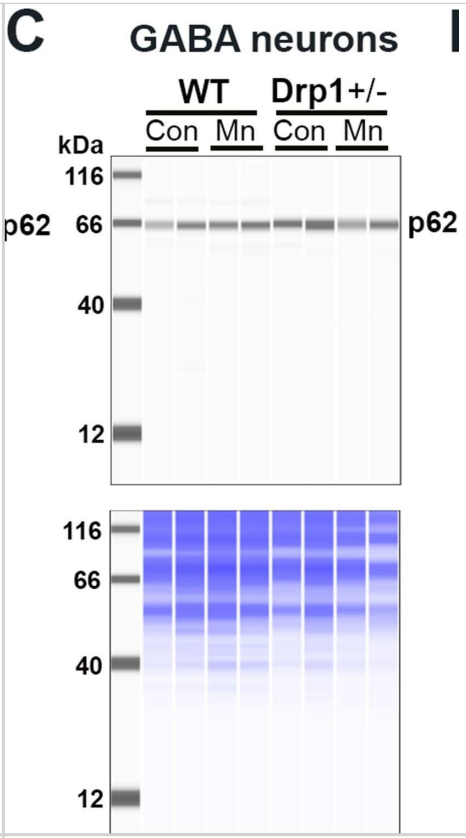
Western Blot: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - p62/SQSTM1 Antibody [NBP1-48320] - Vacuolin-1 analogues identified via virtual screening induced the accumulation of both LC3B-II and SQSTM1 in HeLa cells in a dose dependent manner after a 6 h treatment. Identification of Novel Vacuolin-1 Analogues as Autophagy Inhibitors by Virtual Drug Screening and Chemical Synthesis. *Molecules* (2017)



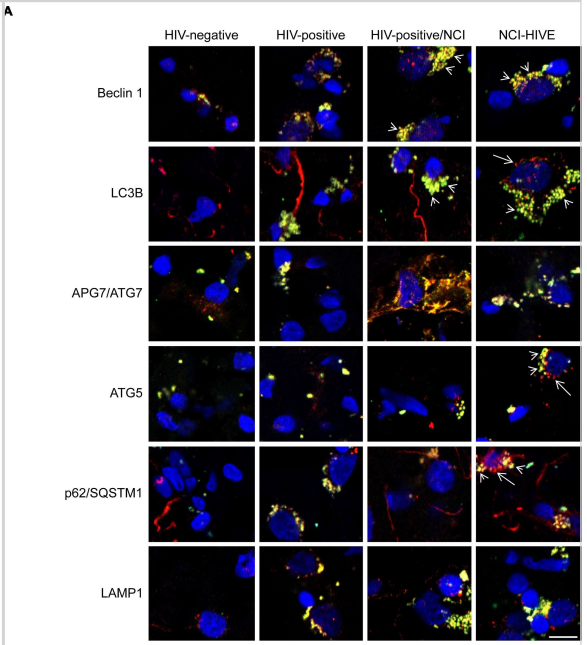
Simple Western: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - Drp1+/- mice is protective against autophagy impairment induced by Mn. (A) Representative images of the coronal mouse midbrain section (20um) co-immunostained for DA neurons (TH, red) in the SNpc and GABA neurons (GAD67, green) in the SNpr. Both of these brain regions were removed by laser microdissection for immunoblotting of p62 (top panels) in DA neurons (B) and GABA neurons (C). Total proteins per lane (bottom panels) were used as loading control. (D) Quantified levels of p62 were significantly increased in DA neurons (P = 0.0013), but not in the GABA neurons (P = 0.5457), of the Mn-treated WT mice. Mn did not significantly increase p62 in DA neurons of the Drp1+/- mice (P = 0.8660) No significant baseline level differences between the two genotypes were observed (P = 0.5664 for TH neurons and P = 0.7675 for GABA neurons. Data represent mean +- SEM, n = 5 for WT control, n = 6 for other groups), two-way ANOVA followed by Tukey post-hoc test. (E) Representative confocal images of mitochondrial morphology of DA neurons after TOM20 immunostaining (upper panels), then skeletonized (middle panels) for subsequent analysis. Scale bar 20um. (F, G) Various parameters of mitochondrial morphology were quantified using Fiji MiNA plugin. Data represents mean +- SEM (n = 6 mice per group), analyzed by one-way ANOVA, followed by Tukey post-hoc test Image collected and cropped by CiteAb from the following publication (https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-024-00708-w), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



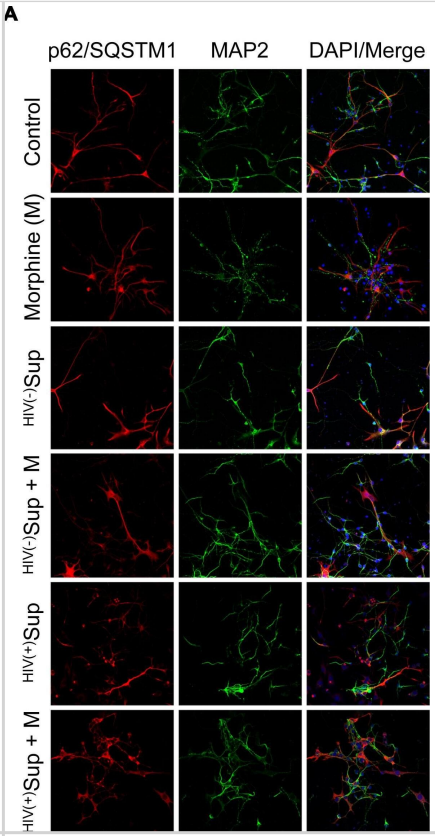
Simple Western: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - Drp1+/- mice is protective against autophagy impairment induced by Mn. (A) Representative images of the coronal mouse midbrain section (20um) co-immunostained for DA neurons (TH, red) in the SNpc and GABA neurons (GAD67, green) in the SNpr. Both of these brain regions were removed by laser microdissection for immunoblotting of p62 (top panels) in DA neurons (B) and GABA neurons (C). Total proteins per lane (bottom panels) were used as loading control. (D) Quantified levels of p62 were significantly increased in DA neurons (P = 0.0013), but not in the GABA neurons (P = 0.5457), of the Mn-treated WT mice. Mn did not significantly increase p62 in DA neurons of the Drp1+/- mice (P = 0.8660) No significant baseline level differences between the two genotypes were observed (P = 0.5664 for TH neurons and P = 0.7675 for GABA neurons. Data represent mean +- SEM, n = 5 for WT control, n = 6 for other groups), two-way ANOVA followed by Tukey post-hoc test. (E) Representative confocal images of mitochondrial morphology of DA neurons after TOM20 immunostaining (upper panels), then skeletonized (middle panels) for subsequent analysis. Scale bar 20um. (F, G) Various parameters of mitochondrial morphology were quantified using Fiji MiNA plugin. Data represents mean +- SEM (n = 6 mice per group), analyzed by one-way ANOVA, followed by Tukey post-hoc test Image collected and cropped by CiteAb from the following publication (https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-024-00708-w), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



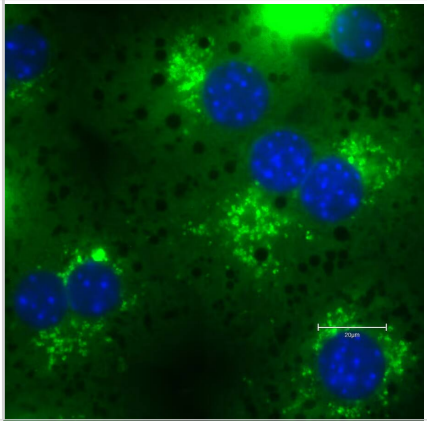
Autophagy associated protein immunoreactivity in HIV-infected brain tissue. (A) Representative images from five randomly selected fields of cells each examined in duplicate frontal lobe white matter sections for the indicated subject groups. The indicated proteins were labeled red & microglia with the cell-type-specific marker Iba1 (green). Blue staining indicates cell nuclei. Arrow heads indicate examples of higher Iba1 immunoreactivity whereas arrows indicate more focal (punctal) vs. diffuse (filamentous) patterns of autophagy associated protein expression. Scale bar = 10 µm. (B) Quantification of relative Iba1 immunoreactivity from (A). F(3,20) = 6.450, p = 0.0031; □p < 0.05 when compared to all other subject groups. Error bars show the SEM for the average values of 2–6 regions from each subject group across the six autophagy associated proteins examined. (C) Quantification of the indicated autophagy associated protein relative immunoreactivity from (A). Beclin 1: F(3,12) = 11.29, p = 0.0008; LC3B: F(3,12) = 1.994, p = 0.1687; APG7/ATG7: F(3,12) = 84.20, p = < 0.0001; ATG5: F(3,12) = 6.218, p = 0.0086; p62/SQSTM1: F(3,12) = 87.04, p = < 0.0001; LAMP1: F(3,12) = 8.317, p = 0.0029. □p < 0.05 when compared to HIV-negative; #p < 0.05 when compared to HIV-positive; & Ωp < 0.05 when compared to HIV-positive/NCI subjects. Error bars show the SEM for four regions from each subject group. Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/Article/10.3389/fmicb.2015.00653/abstract), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: p62/SQSTM1 Antibody - BSA Free [NBP1-48320] - Effects on autophagic activity & dendritic length of neurons exposed to supernatant from HIV-1-infected microglia in combination with morphine. (A) Representative images of neurons with the indicated treatments. Sup, supernatant from uninfected [HIV(-)] & HIV-1-infected [HIV(+)] microglia. Cells were immunolabeled with antibodies to the autophagic activity marker p62/SQSTM1 (red) & the neuronal cell-type-specific marker MAP2 (green). DAPI (blue) staining indicates cell nuclei. (B) Quantification of p62/SQSTM1 immunoreactivity from (A). Data are presented as the percentage of control cells which was set at 100; F(5,24) = 5.882, p = 0.0011; □p < 0.05 when compared to HIV(+)Sup + morphine treatment. (C) Western blotting analysis of p62/SQSTM1 & LAMP1 expression levels for the indicated treatments. GAPDH was used as a loading control. Blots are representative of three independent experiments. (D) Measurement of dendrite length from (A). F(5,24) = 26.15, p = < 0.0001; Φp < 0.05 when compared to morphine; Ψp < 0.05 when compared to HIV(-)Sup; Ωp < 0.05 when compared to HIV(-)Sup + morphine; #p < 0.05 when compared to HIV(+)Sup; & □p < 0.05 when compared to HIV(+)Sup + morphine treatment. Error bars show the SEM for five randomly selected fields totaling at least 100 cells from each group. Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/Article/10.3389/fmicb.2015.00653/abstract), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/Immunofluorescence: Rabbit Polyclonal p62/SQSTM1 Antibody [NBP1-48320] - Mice hepatocytes stained with p62/SQSTM1 Antibody. Image from a verified customer review.



Publications

Dong MZ, Ouyang YC, Gao SC et Al. Protein phosphatase 4 maintains the survival of primordial follicles by regulating autophagy in oocytes Cell Death Dis 2024-09-08 [PMID: 39245708]

Yao L, Zi G, He M et Al. Asparagine endopeptidase regulates lysosome homeostasis via modulating endomembrane phosphoinositide composition Cell Death Dis 2025-01-02 [PMID: 39743643]

Cui S, Zhang T, Xiong X et Al. Detergent-insoluble PFN1 inoculation expedites disease onset and progression in PFN1 transgenic rats Front Neurosci 2023-09-25 [PMID: 37817804]

Navas-Madronal M, Rodriguez C, Kassan M et al. Enhanced endoplasmic reticulum and mitochondrial stress in abdominal aortic aneurysm Clin. Sci. 2019-06-25 [PMID: 31239294]

Yoon Y, Go G, Yoon S Et Al. Melatonin Treatment Improves Renal Fibrosis via miR-4516/SIAH3/PINK1 Axis Cells 2021-07-03 [PMID: 34359852]

Tedeschi V, Nele V, Valsecchi V et al. Nanoparticles Encapsulating Phosphatidylinositol Derivatives Promote Neuroprotection and Functional Improvement via a long-lasting activation of TRPML1 lysosomal channel in Preclinical Models of ALS. Pharmacological research 2024-11-02 [PMID: 39491634]

Amanda JM, Taylor ED, Artem NP et al. Alcohol-induced Golgiphagy is triggered by the downregulation of Golgi GTPase RAB3D Autophagy. 2024-04-09 [PMID: 38591519] (Western Blot, Mouse)

O'Rourke R, Erdemir GA, Huang YA Assays of Monitoring and Measuring Autophagic Flux for iPSC-Derived Human Neurons and Other Brain Cell Types Methods in molecular biology (Clifton, N.J.) 2023-06-10 [PMID: 37300779]

Xianzun Tao, Jiaqi Liu, Zoraida Diaz-Perez, Jackson R. Foley, Ashley Nwafor, Tracy Murray Stewart, Robert A. Casero, R. Grace Zhai Reduction of spermine synthase enhances autophagy to suppress Tau accumulation Cell Death & Disease 2024-05-13 [PMID: 38740758]

Abdalla Elbialy, Mai Kitauchi, Dai Yamanouchi, Anne Vejux Antioxidants and azd0156 Rescue Inflammatory Response in Autophagy-Impaired Macrophages International Journal of Molecular Sciences 2023-12-21 [PMID: 38203340]

Wenting You, Kèvin Knoops, Tos T. J. M. Berendschot, Birke J. Benedikter, Carroll A. B. Webers, Chris P. M. Reutelingsperger, Theo G. M. F. Gorgels PGC-1a mediated mitochondrial biogenesis promotes recovery and survival of neuronal cells from cellular degeneration Cell Death Discovery 2024-04-17 [PMID: 38632223]

Yefimova, M G, LefEvre, C Et al. Granulosa cells provide elimination of apoptotic oocytes through unconventional autophagy-assisted phagocytosis. Hum Reprod 2020-06-01 [PMID: 32531067]

More publications at http://www.novusbio.com/NBP1-48320

Procedures

Western Blot Protocol for p62/SQSTM1 Antibody (NBP1-48320)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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 manufacturer's instructions.

Immunohistochemistry-Paraffin Protocol for p62/SQSTM1 Antibody (NBP1-48320)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for p62/SQSTM1 Antibody (NBP1-48320)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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Products Related to NBP1-48320

H00000644-M01	Biliverdin Reductase A/BLVRA Antibody (4G4-2B6) - Azide and BSA Free
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.

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