Product Datasheet

Ubiquilin 2 Antibody (6H9) - BSA Free
NBP2-25164

Unit Size: 0.1 ml

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

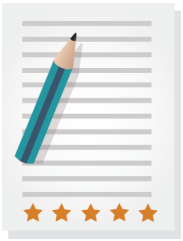
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NBP2-25164

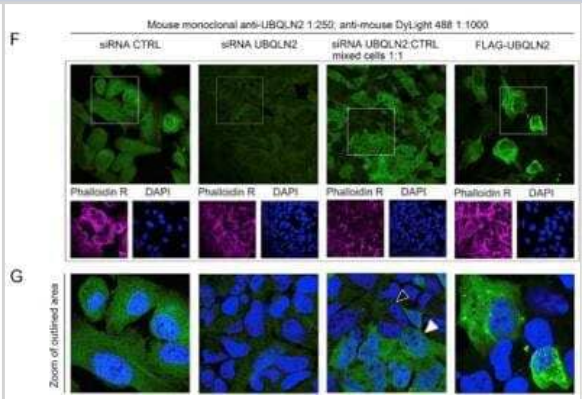
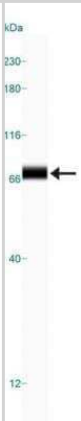
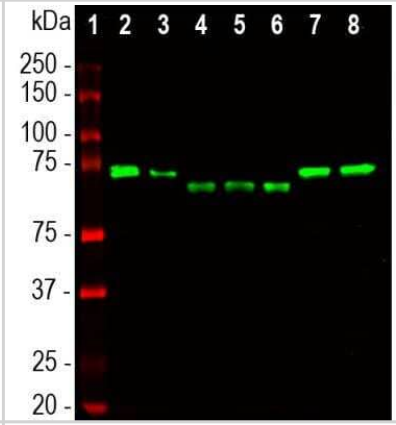
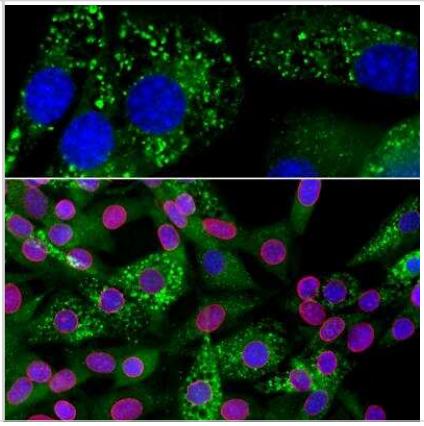
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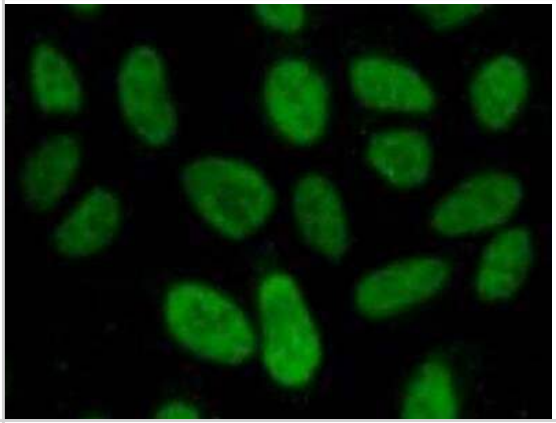
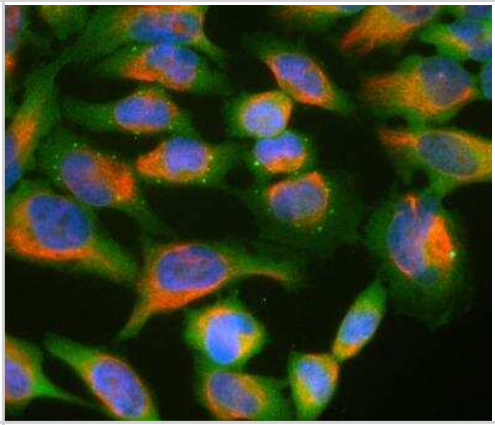
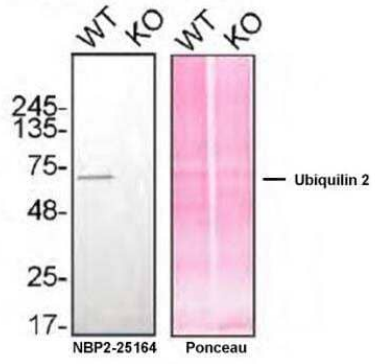
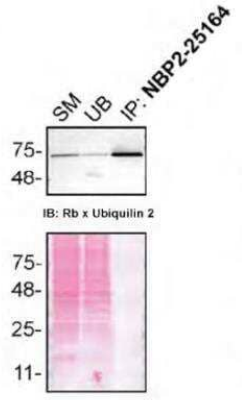
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	6H9
Preservative	5mM Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	50% PBS, 50% glycerol
Target Molecular Weight	66-68 kDa

Product Description	
Description	Novus Biologicals Knockout (KO) Validated Mouse Ubiquilin 2 Antibody (6H9) - BSA Free (NBP2-25164) is a monoclonal antibody validated for use in IHC, WB, ICC/IF, Simple Western and IP. Anti-Ubiquilin 2 Antibody: Cited in 17 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	29978
Gene Symbol	UBQLN2
Species	Human, Mouse, Rat
Immunogen	Human Ubiquilin 2 expressed in and purified from E. coli [UniProt# Q9UHD9]

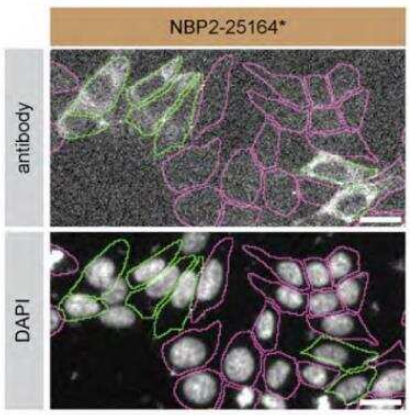
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000-1:2000, Simple Western 1:100, Immunohistochemistry 1:1000, Immunocytochemistry/ Immunofluorescence 1:1000, Immunoprecipitation, Immunohistochemistry-Frozen, Knockout Validated, Knockdown Validated
Application Notes	This Ubiquilin 2 (6H9). antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry, and Western Blot, where a band can be seen at approx. 66-68 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 74 kDa. Use in Immunohistochemistry-Frozen reported in scientific literature (PMID:33028421).

Images

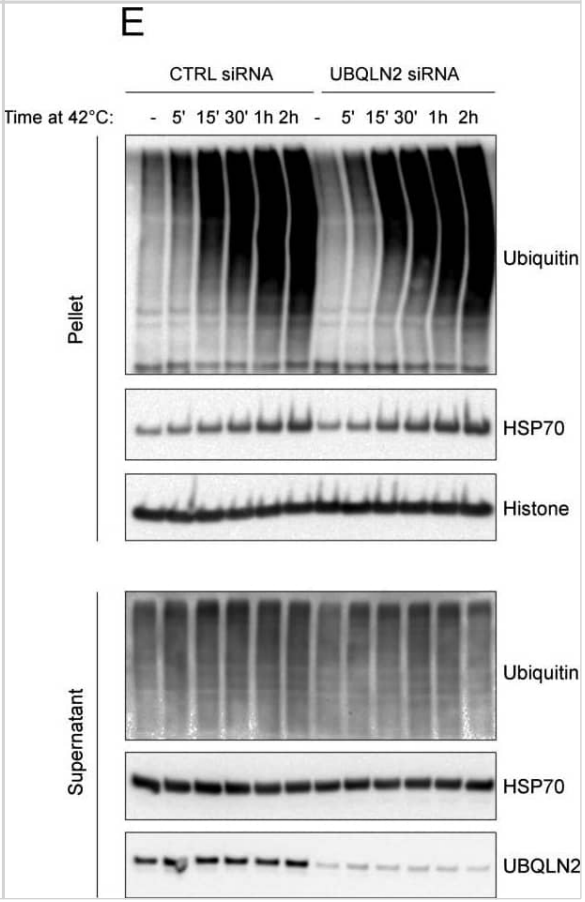
Immunocytochemistry/ Immunofluorescence: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - Validation of UBQLN2 antibody for staining of endogenous UBQLN2 in U2OS cells. U2OS cells were transfected with control siRNA or siRNA targeting UBQLN2. Cells were either seeded as separate groups (i.e., control and UBQLN2 siRNA) or mixed 1:1 and seeded together. Cells were stained using the mouse monoclonal anti-UBQLN2 6H9 (Novus NBP2-25164), at 1:250 in 2% BSA PBS for 1h. Secondary antibody was goat Anti-Mouse DyLight 488. Knockdown of UBQLN2 can be clearly seen to decrease the signal, indicating that the antibody is specific to UBQLN2. (G) displays zoom of the indicated areas in. For the mixed cells a white arrowhead indicates a cell transfected with control siRNA and a black arrowhead a cell transfected with UBQLN2 siRNA. Image collected and cropped by CiteAb from the following publication (https://doi.org/10.1016/j.cell.2016.07.001), licensed under a CC-BY license.	
Simple Western: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - Simple Western lane view shows a specific band for Ubiquilin 2 in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - Analysis of different tissue and cell lysates using mouse mAb to ubiquilin 2, NBP2-25164, dilution 1:1,000 in green: [1] protein standard (red), [2] NIH-3T3, [3] C6, [4] HEK293, [5] HeLa, [6] SH-SY5Y, [7] rat whole brain, and [8] mouse whole brain. The band at 65-70kDa corresponds to ubiquilin 2 protein, which is known to differ between the human and rodent proteins.	
Immunocytochemistry/Immunofluorescence: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - Analysis of an NIH-3T3 cell culture stained with mouse mAb to ubiquilin 2, NBP2-25164, dilution 1:1,000 in green, and costained with chicken pAb to lamin A/C, dilution 1:5,000 in red. The blue is DAPI staining of nuclear DNA. The cells were treated with 50uM of chloroquine, an inhibitor of autophagy, for 16 hours prior to staining. NBP2-25164 antibody reveals vesicular staining of ubiquilin 2 protein accumulated in lysosomes in the cytoplasm, while the lamin A/C antibody stains the nuclear lamina.	

Immunocytochemistry/Immunofluorescence: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - Analysis of Human U2OS cells stained with Ubiquilin 2 antibody. Primary antibody dilution: 1:1000. Image from verified customer review.	
Immunocytochemistry/Immunofluorescence: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - HeLa cell cultures stained with NBP2-25164 (green) and chicken polyclonal antibody to Vimentin: NB300-223 (red). In most individual cells Ubiquilin 2 is present diffusely in the cytoplasm of cells, though some cells show enrichment of the protein in spherical autophagosome-like structure.	
Western Blot: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - Lysates of HAP1 WT and Ubiquilin 2 KO were prepared, and 30 ug of protein were processed for immunoblot with NBP2-25164. The Ponceau stained transfer of the blot is shown. Antibody dilution used: 1/2000. Predicted band size: 65 kDa. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).	
Immunoprecipitation: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - HAP1 lysates were prepared, and immunoprecipitation was performed using 2.0 ug of Ubiquilin 2 antibody (NBP2-25164) pre-coupled to Dynabeads protein G. Samples were washed and processed for immunoblot with NBP2-25164. For immunoblot, Recombinant Ubiquilin 2 (Rb x Ubiquilin 2) was used at 1/1000. The Ponceau stained transfer of the blot is shown. SM=4% starting material; UB=4% unbound fraction. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).	

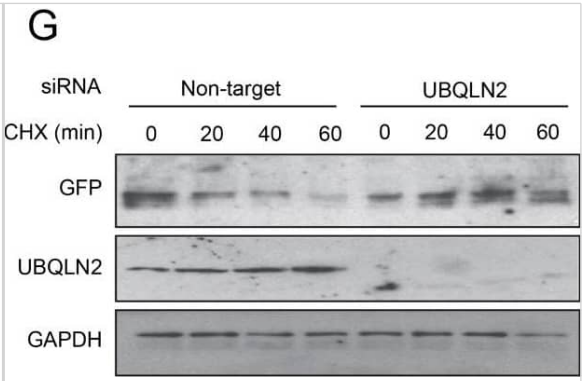
Immunocytochemistry/ Immunofluorescence: Ubiquilin 2 Antibody (6H9) [NBP2-25164] - HAP1 WT and Ubiquilin 2 KO cells were labeled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio in a 96-well plate with glass bottom. Cells were stained with Ubiquilin-2 antibody (NBP2-25164) and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative image of the blue and red (grayscale) channels is shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Antibody dilution used: 1/1000. Bars = 10 um Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).



Heat Shock Generates Insoluble Ubiquitin-Positive Aggregates and Does Not Inactive Proteasomes, Related to Figure 1(A) UBQLN2 is not pelleted when cells are heat shocked post lysis. Cell lysates were incubated at 37 or 42C and then fractionated into soluble (S) and pellet (P) fraction. This indicates that UBQLN2 itself does not aggregate as a result of high temperature.(B) UBQLN2 levels are not upregulated in response to heat shock. HSP70 and GAPDH were used as a positive and negative controls, respectively.(C) Heat shock aggregates are insoluble in up to and including 1% SDS but are solubilized in 2% SDS. Blotting of soluble and pellet fractions with anti-ubiquitin and UQBLN2 antibodies confirmed dissolution of the aggregates in 2% SDS.(D) Proteasomes are active after heat shock. To confirm that proteasome activity was not affected by heat shock, we incubated U2OS and MEFs at the indicted temperatures for 2h. Cells were then harvested and cell lysates were incubated with the proteasome inhibitor MG132 or DMSO, followed by incubation with a fluorescent proteasome-activity probe, as indicated. The presence of fluorescently labeled beta-subunits at the same intensity under both heat stress and normal temperature, indicate that proteasome activity is not significantly affected by heat shock.(E) U2OS cells were treated with control or UBQLN2 siRNA and subjected to heat shock for the indicated times. Analysis of the pellet fraction revealed that insoluble ubiquitylated aggregates are generated within 5 min of heat shock, but that depletion of UBQLN2 does not noticeably alter the accumulation of these aggregates at any of the indicated time points. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27477512), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Puromycin Does Not Upregulate UBQLN2 Levels and UBQLN2 Clears Nuclear Aggregated GFP-u, Related to Figure 2(A) Schematic representation on how the three fractions, total insoluble, nuclear soluble and total insoluble were generated.(B) Cells treated with puromycin were fractionated as indicated and treatment did not induce the nuclear localization of UBQLN2.(C) Puromycin treatment did not induce the upregulation of UBQLN2 protein.(D) Schematic showing the GFPu-NLS construct (Bennett et al., 2005).(E) HEK293 cells stably expressing GFPu-NLS were subject to heat shock for 2h at 42C and fractionated into soluble and insoluble fractions. GFPu-NLS recruited to the insoluble fraction after heat shock indicating its heat-induced aggregation.(F) GFPu-NLS cells were subject to heat shock for 2h at 42C and proteasome inhibition with 25 μM MG132 as indicated. UBQLN2 was immunoprecipitated and GFPu-NLS was found to co-immunoprecipitate only upon heat shock, consistent with UBQLN2 nuclear localization. Combined heat shock and proteasome inhibition increased the binding further.(G) GFPu-NLS cells were depleted of UBQLN2 or treated with a control non-targeting siRNA then treated with 50 μg/ml cycloheximide (CHX) for the indicated time to measure turnover. Turnover was quantified using data from three independent experiments.Error bars represent SE. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27477512), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Mohan, HM;Fernandez, MG;Huang, C;Lin, R;Ryou, JH;Seyfried, D;Grotewold, N;Barget, AJ;Whiteley, AM;Basrur, V;Mosalaganti, S;Paulson, HL;Sharkey, LM; Endogenous retrovirus-like proteins recruit UBQLN2 to stress granules and shape their functional biology Science advances 2025-07-18 [PMID: 40680123]

Almaguer-Mederos L, Kandi A, Sen N et al. Spinal cord phosphoproteome of SCA2 mouse model reveals alteration of ATXN2-N-term PRM-SH3-actin interactome and of autophagy. Molecular & Cellular Proteomics : MCP 2025-09-22 [PMID: 40992657]

Takei T, Sasazawa Y, Noshiro D et al. Ubiquilin-2 liquid droplets catalyze α-synuclein fibril formation. The EMBO Journal 2025-10-14 [PMID: 41087580]

McDowell, I;Ayoubi, R;Fotouhi, M;Southern, K;McPherson, PS;Laflamme, C;NeuroSGC/YCharOS/EDDU collaborative group, ;ABIF Consortium, ; The identification of high-preforming antibodies for Ubiquilin-2 for use in Western Blot, immunoprecipitation, and immunofluorescence F1000Research 2023-03-30 [PMID: 37359784] (Immunocytochemistry/ Immunofluorescence, Mouse)

Teyssou E, Chartier L, Amador MD et al. Novel UBQLN2 mutations linked to amyotrophic lateral sclerosis and atypical hereditary spastic paraplegia phenotype through defective HSP70-mediated proteolysis Neurobiology of Aging 2017-10-01 [PMID: 28716533] (Immunocytochemistry/ Immunofluorescence, Mouse)

Whiteley AM, Prado MA, de Poot SAH et Al. Global proteomics of Ubqln2-based murine models of ALS J Biol Chem 2020-12-10 [PMID: 33277362] (Western Blot)

Laura R. Nementzik, Kyrah M. Thumbadoo, Helen C. Murray, David Gordon, Shu Yang, Ian P. Blair, Clinton Turner, Richard L. M. Faull, Maurice A. Curtis, Catriona McLean, Garth A. Nicholson, Molly E. V. Swanson, Emma L. Scotter Distribution of ubiquilin 2 and TDP □43 aggregates throughout the CNS in UBQLN2 p. T487I □linked amyotrophic lateral sclerosis and frontotemporal dementia Brain Pathology 2023-12-19 [PMID: 38115557]

Dao TP, Rajendran A, Galagedera SKK et al. Short disordered N-termini & proline-rich domain are major regulators of UBQLN1/2/4 phase separation Biophysical journal 2023-11-30 [PMID: 38041404] (WB)

Dao TP, Rajendran A, Galagedera SKK et al. Short N-terminal disordered regions and the proline-rich domain are major regulators of phase transitions for full-length UBQLN1, UBQLN2 and UBQLN4 bioRxiv : the preprint server for biology 2023-09-29 [PMID: 37808720] (WB)

Mohan HM, Trzeciakiewicz H, Pithadia A et al. RTL8 promotes nuclear localization of UBQLN2 to subnuclear compartments associated with protein quality control Cellular and molecular life sciences : CMLS 2022-03-05 [PMID: 35247097] (ICC/IF, WB, Human)

Nementzik L, Thumbadoo K, Murray H et al. Distribution of ubiquilin 2 and TDP-43 aggregates throughout the CNS inUBQLN2p.T487I-linked amyotrophic lateral sclerosis and frontotemporal dementia bioRxiv 2023-02-10 (IHC, Human)

Buel GR, Chen X, Myint W et al. E6AP AZUL interaction with UBQLN1/2 in cells, condensates, and an AlphaFold-NMR integrated structure Structure (London, England : 1993) 2023-02-14 [PMID: 36827983]

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NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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