Product Datasheet

Ubiquitin Antibody
NB300-129

Unit Size: 0.05 ml

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

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Publications: 12

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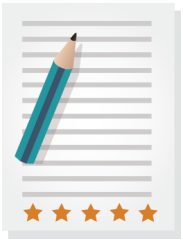
Updated 10/23/2024 v.20.1



NB300-129

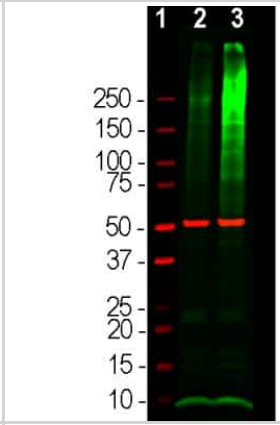
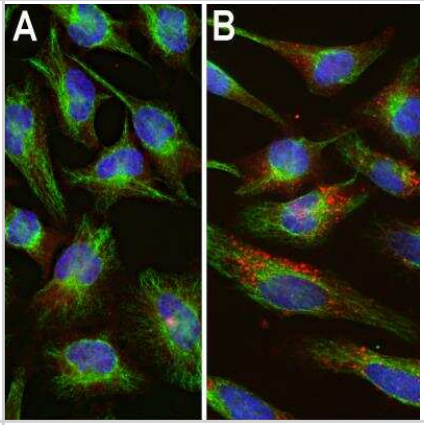
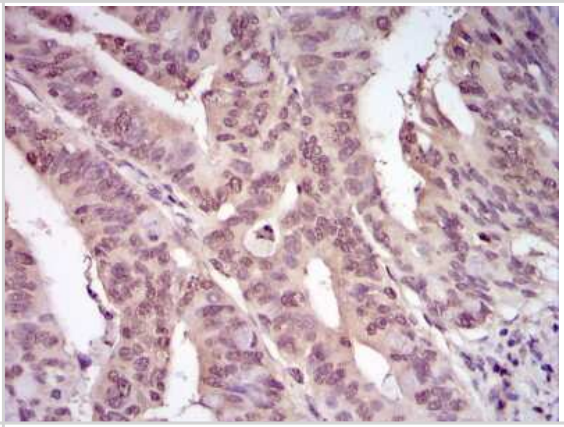
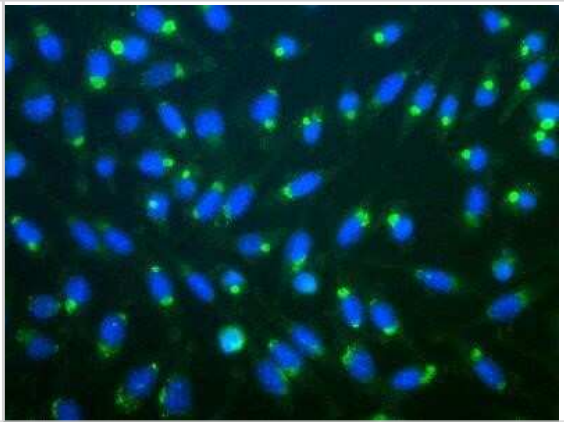
Ubiquitin Antibody

Product Information	
Unit Size	0.05 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.035% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Supplied as serum
Target Molecular Weight	8.5 kDa

Product Description	
Host	Rabbit
Gene ID	6233
Gene Symbol	RPS27A
Species	Human, Mouse, Rat
Specificity/Sensitivity	This antibody recognizes ubiquitinated inclusion bodies and both the mono- and polyubiquitin forms. This recognizes aggresomes.
Immunogen	Purified ubiquitin conjugated with glutaraldehyde to KLH

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Proximity Ligation Assay
Recommended Dilutions	Western Blot 1:5000-1:10000, Immunohistochemistry 1:500-1:1000, Immunocytochemistry/ Immunofluorescence 1:500-1:1000, Immunohistochemistry-Paraffin 1:500-1:1000, Immunohistochemistry-Frozen 1:500-1:1000, Proximity Ligation Assay
Application Notes	This Ubiquitin antibody can be used for Immunocytochemistry/Immunofluorescence, Immunohistochemistry, and Western blot. IHC-Fr reported in scientific literature (PMID: 25898785). Use in Proximity ligation assay reported in scientific literature (PMID:33053339).

Images

Western Blot: Ubiquitin Antibody [NB300-129] - Analysis of HEK293 cell lysates using rabbit Ubiquitin antibody, dilution 1:5000 (Green), and mouse beta-Tubulin antibody, dilution 1:10000 (Red) used as a loading control. [1] protein standard (Red), [2] cells maintained in normal medium, [3] cells treated with proteasome inhibitor lactacystin (Lc) at 10uM for 16 hours. Lysed cells were lysed and the lysate subjected to electrophoresis on a 4-20% SDS-PAGE gel, then electrophoretically transferred to PVDF membranes. The smear detected above the 200kDa standard represents accumulations of ubiquitinated proteins in the Lc treated cells. The prominent band at ~8kDa corresponds to monoubiquitin.	
Immunocytochemistry/Immunofluorescence: Ubiquitin Antibody [NB300-129] - Analysis of HeLa cells stained with rabbit pAb to ubiquitin, NB300-129, dilution 1:1,000 in red, and costained with chicken pAb to vimentin, dilution 1:10,000, in green. The blue is DAPI staining of nuclear DNA. [A] Control HeLa cells maintained in normal medium, [B] HeLa cells treated with 10uM of the proteasome inhibitor lactacystin (Lc) for 24 hours. Proteasomal inhibition leads to formation of strongly ubiquitin positive cytoplasmic inclusions. Note the diffuse cytoplasmic ubiquitin staining in control cells and well defined ubiquitin positive inclusions in the Lc treated cells.	
Immunohistochemistry-Paraffin: Ubiquitin Antibody [NB300-129] - IHC staining of Ubiquitin in human rectal cancer using DAB with hematoxylin counterstain.	
Immunocytochemistry/Immunofluorescence: Ubiquitin Antibody [NB300-129] - Immunofluorescent staining of aggresomes of mouse L-cells treated with lactacysteine, using NB300-129.	

Publications

W El Manaa, E Duplan, T Goiran, I Lauritzen, L Vaillant B, S Lacas-Gerv, VA Morais, H You, L Qi, M Salazar, U Ozcan, M Chami, F Checler, C Alves da C Transcription- and phosphorylation-dependent control of a functional interplay between XBP1s and PINK1 governs mitophagy and potentially impacts Parkinson disease pathophysiology Autophagy, 2021-05-24;0(0):1-23. 2021-05-24 [PMID: 34030589]

Reichelt J, Sachs W, Frömbling S et al. Non-functional ubiquitin C-terminal hydrolase L1 drives podocyte injury through impairing proteasomes in autoimmune glomerulonephritis Nature communications 2023-04-13 [PMID: 37055432] (ICC/IF, Mouse)

Panicker N, Kam TI, Wang H et al. Neuronal NLRP3 is a parkin substrate that drives neurodegeneration in Parkinson's disease Neuron 2022-05-25 [PMID: 35654037] (WB, Mouse)

Garcia LR, Tenev T, Newman R et al. Ubiquitylation of MLKL at lysine 219 positively regulates necroptosis-induced tissue injury and pathogen clearance Nature communications 2021-06-07 [PMID: 34099649] (Mouse)

Lin LL, Kost ER, Lin CL et al. PAI-1-Dependent Inactivation of SMAD4-Modulated Junction and Adhesion Complex in Obese Endometrial Cancer Cell Rep 2020-10-13 [PMID: 33053339] (PLA, Human)

Sachs W, Sachs M, KrUger E et al. Distinct Modes of Balancing Glomerular Cell Proteostasis in Mucolipidosis Type II and III Prevent Proteinuria J. Am. Soc. Nephrol. 2020-07-08 [PMID: 32641396] (ICC/IF, Mouse)

Llombart V, Trejo SA, Bronsoms S et al. Profiling and identification of new proteins involved in brain ischemia using MALDI-imaging-mass-spectrometry. J Proteomics. 2016-11-22 [PMID: 27888142] (IF/IHC, Mouse)

Munoz-Saez E, de Munck Garcia E, Arahuetes Portero RM et al. Analysis of B-N-methylamino-l-alanine (L-BMAA) neurotoxicity in rat cerebellum. Neurotoxicology. 2015-04-18 [PMID: 25898785] (WB, IHC-Fr, Rat)

Yanagawa, T et al. Regulation of Phosphoglucose Isomerase/Autocrine Motility Factor Activities by the Poly(ADP-Ribose) Polymerase Family-14. Cancer Research 67: 8682-8689. 2007-01-01 [PMID: 17875708] (WB, Human)

Iwahashi, CK et al. Protein composition of the intranuclear inclusion of FXTAS. Brain 129:256-271. 2006-01-01 [PMID: 16246864] (ICC/IF, Human)

Garcia Arocena, D et al. Induction of inclusion formation disruption of lamin A/C sturcture by premutation CGG-repeat RNA in human cultured neural cells. Hum Mol Genet 14:2661-2671. 2005-01-01 [PMID: 16239243] (ICC/IF, Human)

Hoem G, Raske CR, Garcia-Arocena D et al. CGG-repeat length threshold for FMR1 RNA pathogenesis in a cellular model for FXTAS. Hum Mol Genet. 2011-03-09 [PMID: 21389081] (IF/IHC, Human)

Procedures

Immunohistochemistry-Paraffin Protocol Specific for NB300-129: Ubiquitin Antibody
Ubiquitin Antibody:
Materials

1) 1 Phosphate buffered saline (pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na2HPO4 4.3mmol/L, KH2PO4 1.4 mmol/L
2) Citrate buffer, 0.01 M, pH6.0, Sodium Citrate 3g, Citric acid 0.4g
3) 3% Hydrogen peroxide
4) Primary antibody
5) Blocking serum (normal serum)
6) Biotinylated secondary antibody
7) DAB staining kit

Methods

1. Dewax and hydration of slides using xylene and EtOH:
Dry slides for 20 min in a 60 C oven
Add Xylene, 2 x 10 min
100%, 95%, 80%, and 70% EtOH, 5 min each EtOH concentration
Rinse in PBS, 5'

2 Antigen retrieval method (only for paraffin slides)
1a. High-pressure antigen retrieval procedure (recommended method)
Place slides in a glass slide holder (ensure that the slide holder is completely filled with slides, slides without sections if necessary, to ensure even heating. The entire slide holder is immersed in 1000 ml of Citrate buffer (0.01M, pH6.0) within a pressure cooker
Once steam is produced, and ONLY when steam is visible, from the pressure cooker (usually 15-20 min), the required high-pressure will have been reached, and slides will be incubated for 2 min.
Turn off heat, and allow buffer and slides to cool to room temperature
Slides are then rinsed in PBS for 5 minutes
2. Add 3% hydrogen peroxide solution, 10'at RT, then PBS, 3X5'
3. Normal blocking serum, 20'at RT
4. Incubate with Primary Ab, 4C overnight or 1.5 hours at 37C
5. Rinse with PBS, 3 X 5' each rinse
6. Add Biotin-conjugated second antibody, 10'at RT
7. Rinse with PBS, 3 X 5' each rinse
8. Add Streptavidin-Peroxidase, 10'at RT
9. Rinse with PBS, 3 X 5' each rinse
10. Staining with DAB solution, 2-5'under microscope
11. Stop the reaction by washing in tap water
12. Counterstain in Haematoxylin for 3-5 minutes
13. 75%, 80%, 95% and 100% ethanol, 5x2', xylene 2 x 10'

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Products Related to NB300-129

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP2-58398PEP	Ubiquitin Recombinant Protein Antigen

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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