Product Datasheet

alpha Tubulin Antibody (YL1/2) - BSA Free
NB600-506

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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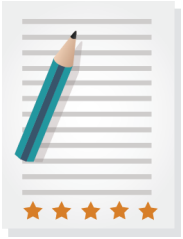
Reviews: 4 Publications: 57

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB600-506

Updated 10/23/2024 v.20.1



NB600-506

alpha Tubulin Antibody (YL1/2) - BSA Free

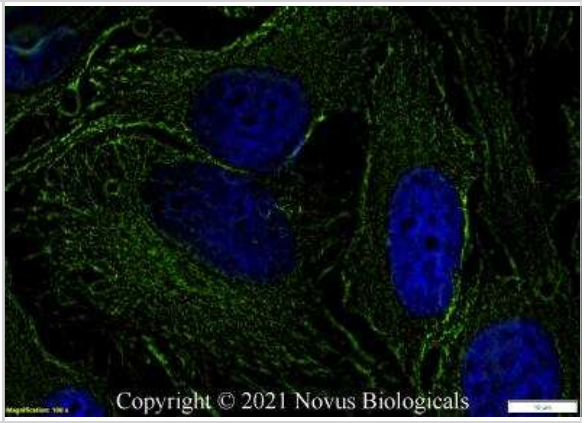
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	YL1/2
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	50 kDa

Product Description	
Host	Rat
Gene ID	7846
Gene Symbol	TUBA1A
Species	Human, Mouse, Rat, Avian, C. elegans, Drosophila, Invertebrate, Mammal, Monkey, Primate, Yeast
Reactivity Notes	S. cerevisiae, S. pombe, Slime molds, Allium. Other species have not been tested. Expected to react with most eukaryotes due to sequence identity. Drosophila reactivity reported in scientific literature (PMID: 24019759). C. elegans reactivity reported in scientific literature (PMID: 29118344). Toxoplasma gondii reactivity reported by customer review.
Marker	Microtubule Marker
Immunogen	This alpha Tubulin Antibody (YL1/2) was developed against full length native protein (purified) (S. cerevisiae).

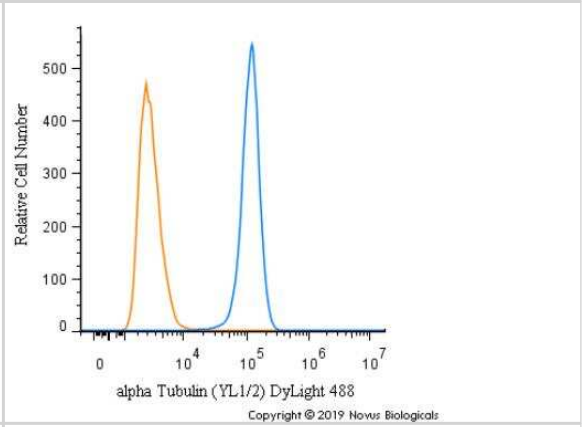
Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Functional, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Radioimmunoassay
Recommended Dilutions	Western Blot 1:5000-1:10000, Flow Cytometry 2-5 ug/0.1x10^6 cells, ELISA 1:100-1:1000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:1000-1:10000. Use reported in scientific literature (PMID 28001364), Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 1:200, Functional reported in scientific literature (PMID 31358662), Radioimmunoassay
Application Notes	NB600-506 is ideal for use as a Western blot loading control, where a band can be seen around 50-55 kDa and as a cytoskeletal marker in Immunocytochemistry.

Images

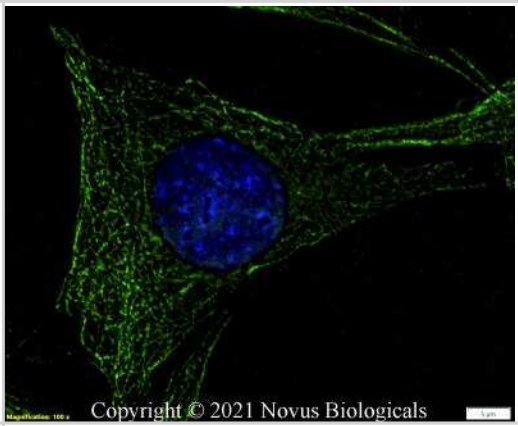
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - HeLa cells were fixed and permeabilized for 10 minutes with -20C MeOH. The cells were incubated with alpha Tubulin Antibody [YL1/2] (NB600-506) at 1ug/ml overnight at 4C and detected with an anti-rat DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



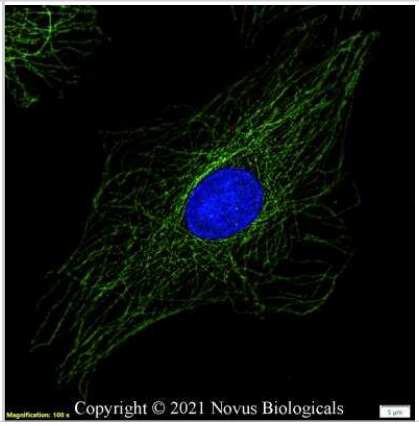
Flow Cytometry: alpha Tubulin Antibody (YL1/2) [NB600-506] - An intracellular stain was performed on SH-SY5Y cells with alpha Tubulin (YL1/2) Antibody NB600-506G (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 488.

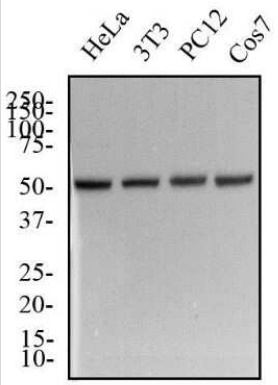
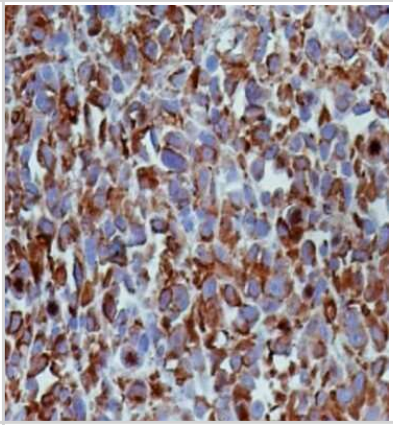
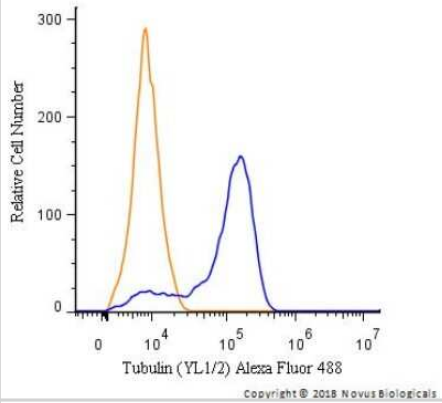
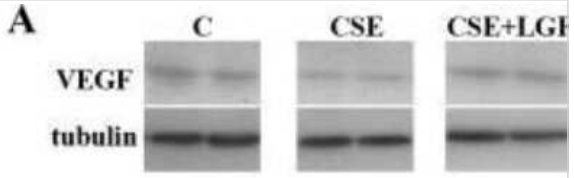


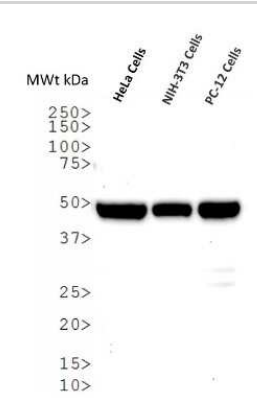
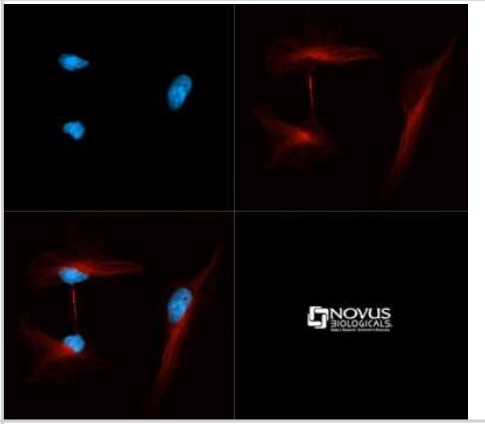
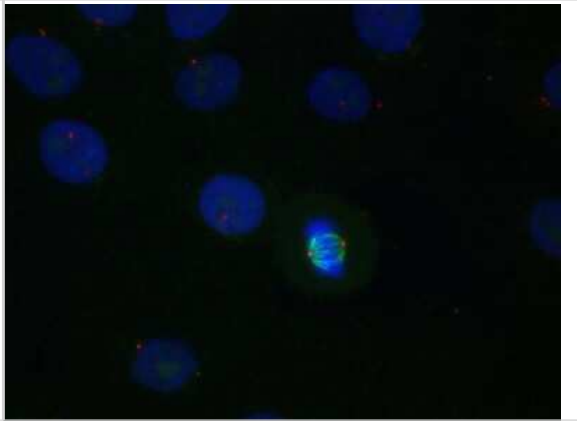
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - NIH3T3 cells were fixed and permeabilized for 10 minutes with -20C MeOH. The cells were incubated with alpha Tubulin Antibody [YL1/2] (NB600-506) at 1ug/ml overnight at 4C and detected with an anti-rat DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



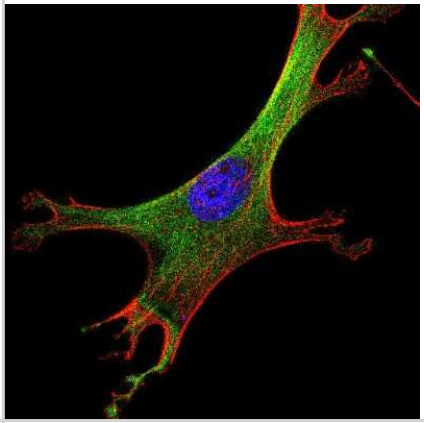
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - Rat FR cells were fixed and permeabilized for 10 minutes with -20C MeOH. The cells were incubated with alpha Tubulin Antibody [YL1/2] (NB600-506) at 1ug/ml overnight at 4C and detected with an anti-rat DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



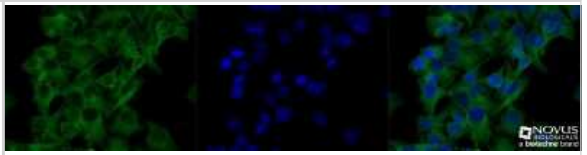
Western Blot: alpha Tubulin Antibody (YL1/2) [NB600-506] - Total protein from human HeLa, mouse 3T3, rat PC12 and African green monkey Cos7 cell lines was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with a 1:6000 dilution of anti-Tubulin (YL 1/2) in 1% non-fat milk in TBST and detected with an anti-rat HRP secondary antibody using chemiluminescence. Alpha tubulin molecular weight: 50 kDa.	
Immunohistochemistry-Paraffin: alpha Tubulin Antibody (YL1/2) [NB600-506] - Tubulin antibody was tested in human breast cancer xenograft using DAB with hematoxylin counterstain.	
Flow Cytometry: alpha Tubulin Antibody (YL1/2) [NB600-506] - An intracellular stain was performed on PC12 cells with Tubulin [YL1/2] Antibody NB600-506AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.	
Western Blot: alpha Tubulin Antibody (YL1/2) [NB600-506] - Tissue levels of VEGF and PCNA.VEGF levels were estimated by western blot. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0112995), licensed under a CC-BY license.	

Western Blot: alpha Tubulin Antibody (YL1/2) [NB600-506] - Western blot analysis of alpha Tubulin (molecular weight: 50 kDa) expression in 1) HeLa, 2) NTERA-2, 3) A431, 4) HepG2, 5) MCF7, 6) NIH-3T3, 7) PC-12 and 8) Cos 7 whole cell lysates using NB600-506.	
Western Blot: alpha Tubulin Antibody (YL1/2) [NB600-506] - Western Blot analysis of whole cell lysates of HeLa, NIH-3T3 and PC-12 cell lines using Tubulin antibody (clone YL1/2). Alpha tubulin molecular weight: 50 kDa.	
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - Tubulin YL1/2 antibody was tested in HeLa cells with Dylight 550 (red). Nuclei were counterstained with DAPI (blue).	
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - B-Tubulin staining (488) and Pericentrin (594) in Mitotic Cell. Image from verified customer review.	

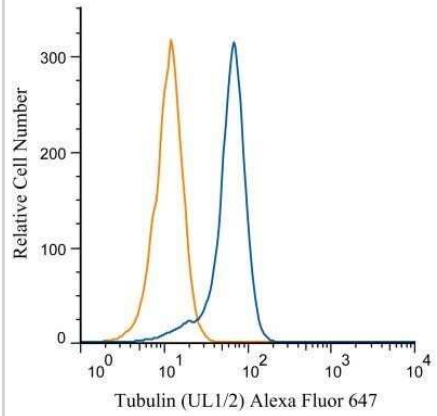
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - IF Confocal analysis of NIH/3T3 cells using Tubulin antibody (NB600-506, 1:5). An Alexa Fluor 488-conjugated Goat to rat IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).



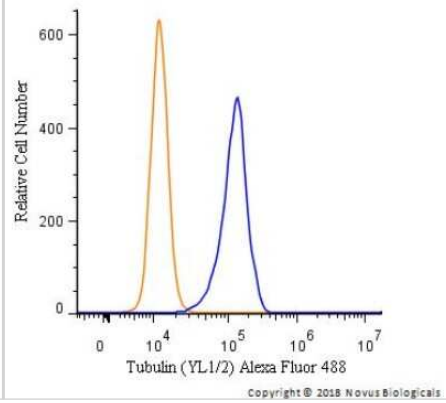
Immunocytochemistry/Immunofluorescence: alpha Tubulin Antibody (YL1/2) [NB600-506] - HeLa cells were fixed and permeabilized for 10 minutes using -20C MeOH. The cells were incubated with anti-Tubulin (YL1/2) at a 1:200 dilution overnight at 4C and detected with an anti-rat Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



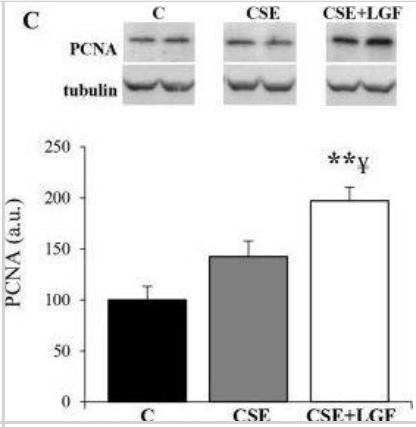
Flow Cytometry: alpha Tubulin Antibody (YL1/2) [NB600-506] - Analysis of Alexa Fluor (R) 647 conjugate of NB600-506. An intracellular stain was performed on HeLa cells with Tubulin antibody (YL1/2) NB600-506AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



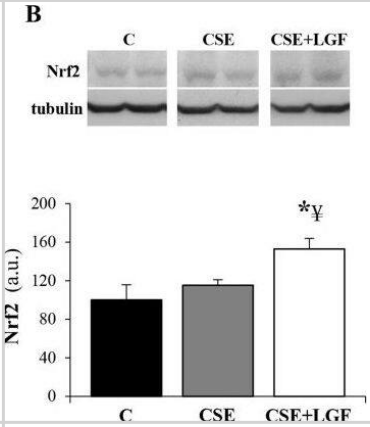
Flow Cytometry: alpha Tubulin Antibody (YL1/2) [NB600-506] - An intracellular stain was performed on NIH3T3 cells with Tubulin [YL1/2] Antibody NB600-506AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



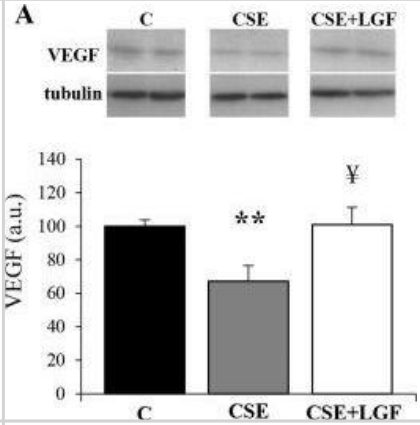
Western Blot: alpha Tubulin Antibody (YL1/2) - BSA Free [NB600-506] - Tissue levels of VEGF & PCNA.VEGF levels were estimated by (A) western blot & (B) ELISA. (C) PCNA levels estimated by western blot. Data were normalized with tubulin. (D) Representative images of PCNA immunohistochemistry staining in the nucleus is shown for CS-exposed mice (CSE) & CS-exposed & LGF-treated mice (CSE+LGF). Scale bars= 50 µm. LGF promoted an increase of PCNA+ cells as showed in the bar graph. ** P<0.01 vs. air-exposed mice; ¥ P<0.05 vs. CS-exposed mice (n =5 per group). Data are presented as mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25401951), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



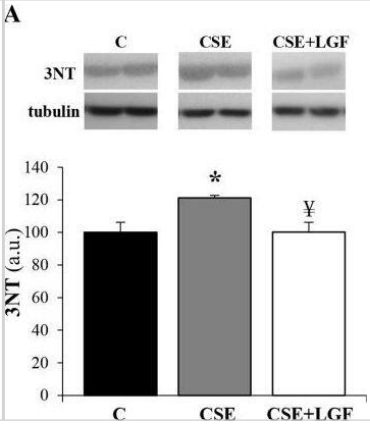
Western Blot: alpha Tubulin Antibody (YL1/2) - BSA Free [NB600-506] - LGF ameliorates oxidative stress.Bars represent (A) 3NT & (B) Nrf2 levels estimated by western blot. Data were normalized with tubulin. * P<0.05 vs. air-exposed mice; ¥ P<0.05 vs. CS-exposed mice (n=5 per group). Data are presented as mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25401951), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



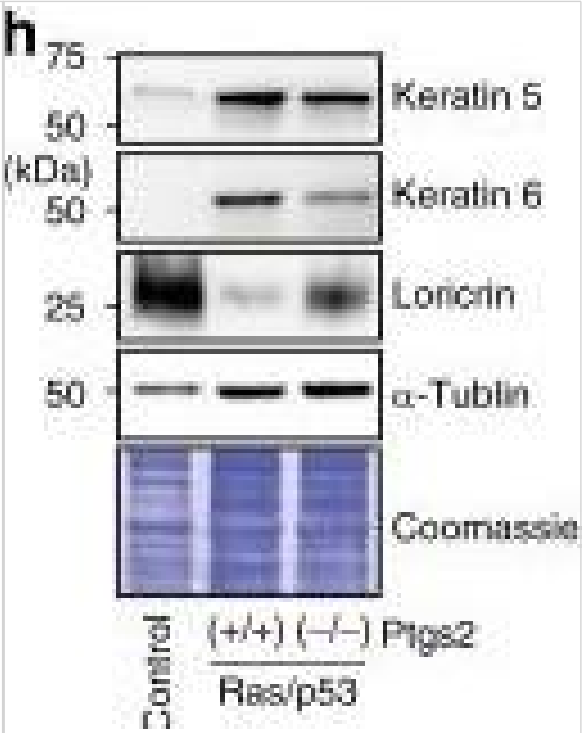
Western Blot: alpha Tubulin Antibody (YL1/2) - BSA Free [NB600-506] - Tissue levels of VEGF & PCNA.VEGF levels were estimated by (A) western blot & (B) ELISA. (C) PCNA levels estimated by western blot. Data were normalized with tubulin. (D) Representative images of PCNA immunohistochemistry staining in the nucleus is shown for CS-exposed mice (CSE) & CS-exposed & LGF-treated mice (CSE+LGF). Scale bars= 50 µm. LGF promoted an increase of PCNA+ cells as showed in the bar graph. ** P<0.01 vs. air-exposed mice; ¥ P<0.05 vs. CS-exposed mice (n =5 per group). Data are presented as mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25401951), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



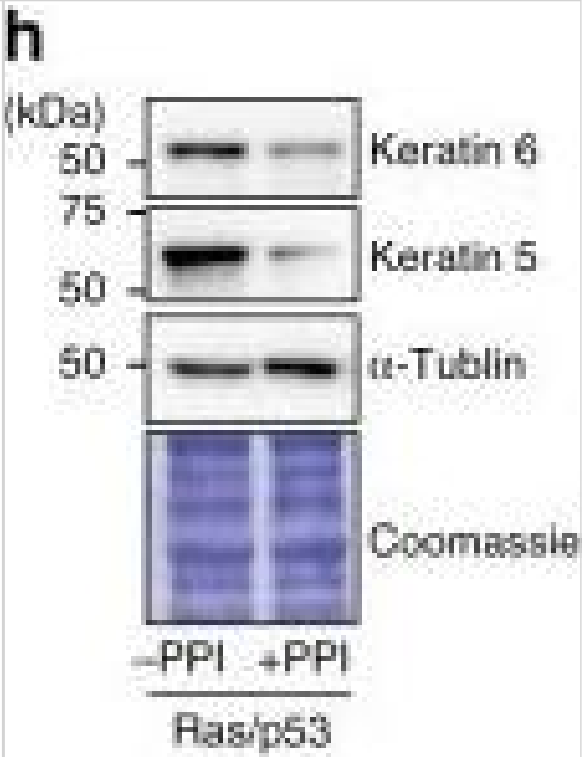
Western Blot: alpha Tubulin Antibody (YL1/2) - BSA Free [NB600-506] - LGF ameliorates oxidative stress.Bars represent (A) 3NT & (B) Nrf2 levels estimated by western blot. Data were normalized with tubulin. * P<0.05 vs. air-exposed mice; ¥ P<0.05 vs. CS-exposed mice (n=5 per group). Data are presented as mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25401951), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: alpha Tubulin Antibody (YL1/2) - BSA Free [NB600-506] - Role of cell-type-specific cyclooxygenase-2 (Cox-2) expression in Ras/p53-mediated tumor formation. a Experimental scheme. b Microscopic phenotype & histology of three-dimensional (3D) organoids with/without celecoxib treatment. c Relative 3D organoid formation was determined by the number of organoids larger than 100 µm, n = 3 independent experiments, 25 fields at high power field (HPF) per sample. Veh, vehicle control; C25, 25 µM; C50, 50 µM celecoxib. d Experimental scheme. e Ras/p53-mediated tumor incidence with/without condition Cox-2 knockout. Ptgs2wt/wt, n = 15 animals; Ptgs2flox/flox, n = 14 animals. f, g Histological phenotypes. h–k Immunoblotting of Keratin5 (Krt5), Krt6 & Loricrin (Lor) demonstrated decreased tumor susceptibility but increased differentiation status, n = 4 independent experiments. l Summary of the contribution of physiological stress factors in the susceptibility of tumor development from tumor-competent Krt15+ progenitors. Data with bar graphs are represented as mean ± SEM. Statistical significance was determined by pair-wise comparison using t test; ns = not significant, *p < 0.05, **p < 0.005. Scale bars, 100 µm. Ptgs2 prostaglandin-endoperoxide synthase 2 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31110179), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: alpha Tubulin Antibody (YL1/2) - BSA Free [NB600-506] - Microenvironmental acidic stressors & tumor susceptibility. a Experimental scheme. Epithelial cells isolated from the regions adjacent or distant from squamocolumnar junction (SCJ). b Microscopic phenotype of three-dimensional (3D) organoids from control & Krt15-CrePR; LSL-KrasG12D; p53flox/flox mice. Histology of 3D organoids demonstrated that oncogenic Ras/p53 increased abnormal growth features. c–e Quantification of relative organoid formation (n = 3 independent experiments, 25 fields at high power field (HPF) per sample) & size distribution (n ≥ 150) from control & experimental mice expressing oncogenic Ras/p53 combination. f Experimental scheme. Phosphate-buffered saline (PBS) (vehicle) or PPI were daily treated by intraperitoneal (i.p.) injections (5 consecutive days per week). g Tumor incidence from Krt15-CrePR; LSL-KrasG12D; p53flox/flox mice, with/without PPI treatment, n = 9 animals per each. Statistical significance was determined by Fisher's exact test; *p < 0.05. h–j Immunoblotting of Keratin5 (Krt5) & Krt6 demonstrated relatively less tumor formation in the PPI treatment group, n = 5 independent experiments. k–m Immunostaining of Krt5, Krt6, & phospho-histone H3 (ph-H3), & histology demonstrated suppressed tumor formation by daily PPI treatment in Krt15-CrePR; LSL-KrasG12D; p53flox/flox mice. PPI, proton pump inhibitor. Data with bar graphs are represented as mean ± SEM. Statistical significance was determined by pair-wise comparison using t test; *p < 0.05, **p < 0.005. Scale bars, 100 µm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31110179), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Jessica Neville Little, Noelle D Dwyer p53 deletion rescues lethal microcephaly in a mouse model with neural stem cell abscission defects Human Molecular Genetics 2020-02-01 [PMID: 30304535]

Parajuli S, Tealsey DC, Murali B et al. Human Ribonuclease H1 resolves R loops and thereby enables progression of the DNA replication fork J. Biol. Chem. 2017-07-17 [PMID: 28717002]

Gabriella S. Darmasaputra, Cindy C. Geerlings, Susana M. Chuva de Sousa Lopes, Hans Clevers, Matilde Galli Binucleated human hepatocytes arise through late cytokinetic regression during endomitosis M phase The Journal of Cell Biology 2024-08-05 [PMID: 38727809]

Dingxi Zhou, Mariana Borsa, Daniel J. Puleston, Susanne Zellner, Jesusa Capera, Sharon Sanderson, Martina Schifferer, Svenja S. Hester, Xin Ge, Roman Fischer, Luke Jostins, Christian Behrends, Ghada Alsaleh, Anna Katharina Simon Mapping autophagosome contents identifies interleukin-7 receptor-α as a key cargo modulating CD4+ T cell proliferation Nature Communications 2022-09-02 [PMID: 36055998]

Kawakami M, Mustachio LM, Chen Y et al. A Novel CDK2/9 Inhibitor CYC065 Causes Anaphase Catastrophe and Represses Proliferation, Tumorigenesis, and Metastasis in Aneuploid Cancers Molecular Cancer Therapeutics 2021-03-01 [PMID: 33277443] (Immunocytochemistry/ Immunofluorescence)

Onuma TA, Hayashi M, Gyoja F et al. A chordate species lacking Nodal utilizes calcium oscillation and Bmp for left-right patterning Proceedings of the National Academy of Sciences 2020-02-25 [PMID: 32029598]

Kobayashi Y, Tomoshige S, Imakado K et al. Ciliary GPCR-based transcriptome as a key regulator of cilia length control FASEB BioAdvances 2021-09-01 [PMID: 34485842] (Block/Neutralize)

Moorthy BT, Jiang C, Patel DM et al. The evolutionarily conserved arginyltransferase 1 mediates a pVHL-independent oxygen-sensing pathway in mammalian cells Developmental Cell 2022-03-04 [PMID: 35247316]

Nakazato Y, Otaki JM Protein Delivery to Insect Epithelial Cells In Vivo: Potential Application to Functional Molecular Analysis of Proteins in Butterfly Wing Development Biotech (Basel (Switzerland)) 2023-04-16 [PMID: 37092472]

Dushku EGavriilidis M A transgenic macrophage-based platform to assess the efficacy and specificity of HuR modulators in inflammation Journal of Biological Research 2023-01-01

Darmasaputra G, Chuva de Sousa Lopes S, Clevers H, Galli M Binucleated human hepatocytes arise through loss of membrane anchorage to the midbody during endomitosis bioRxiv 2023-04-17 (ICC/IF, Human)

Details:
Dilutions: 1:1000

Courtney A, Liegey J, Burke N Et al. Characterization of geometric variance in the epithelial nerve net of the ctenophore Pleurobrachia pileus J Comp Neurol 2021-12-21 [PMID: 34933399]

Details:
Citation using the DyLight 488 version of this antibody.

More publications at http://www.novusbio.com/NB600-506

Procedures

Western Blot Protocol for alpha Tubulin Antibody (NB600-506)
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.

Flow (Intracellular) Protocol for alpha Tubulin Antibody (NB600-506)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunocytochemistry/ Immunofluorescence Protocol for alpha Tubulin Antibody (NB600-506)
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.

Immunohistochemistry-Paraffin Protocol for alpha Tubulin Antibody (NB600-506)
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.

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Products Related to NB600-506

HAF005	Goat anti-Rat IgG Secondary Antibody [HRP]
NBP1-75398	Goat anti-Rat IgG (H+L) Secondary Antibody (Pre-adsorbed)
NBP2-21947-0.1mg	Rat IgG2a Isotype Control (2A3)
NB600-506G	alpha Tubulin Antibody (YL1/2) [DyLight 488]

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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