Product Datasheet

beta Tubulin Antibody - BSA Free
NB600-936

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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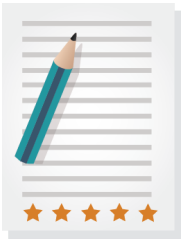
Updated 2/17/2025 v.20.1



NB600-936

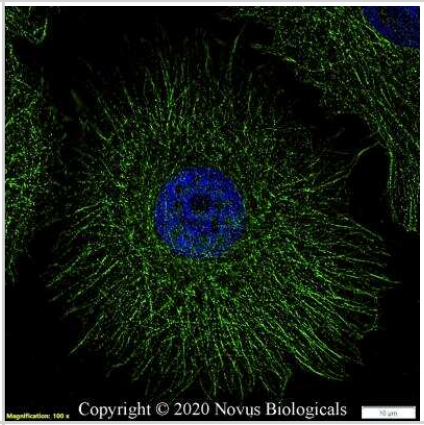
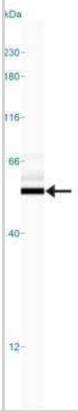
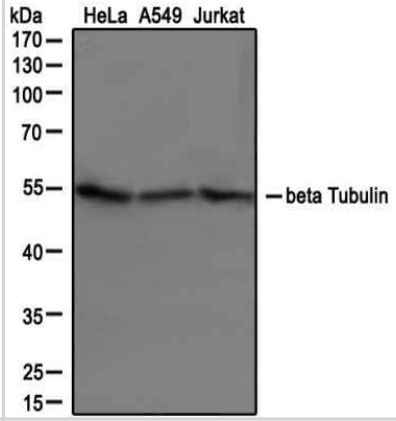
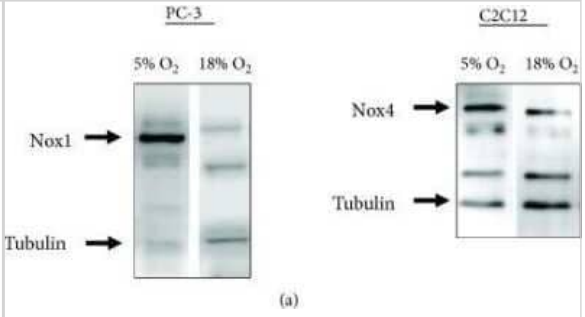
beta Tubulin Antibody - BSA Free

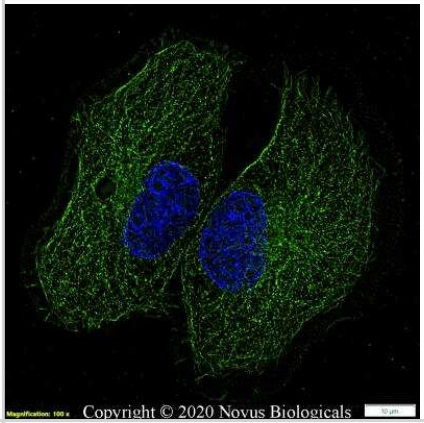
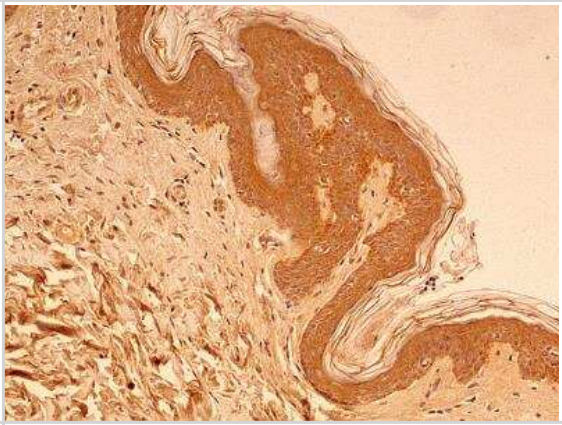
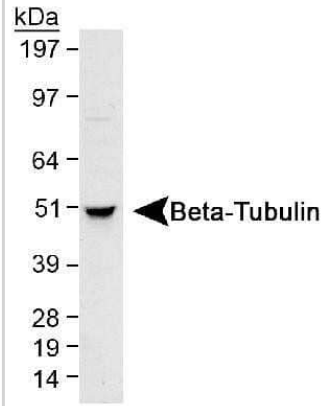
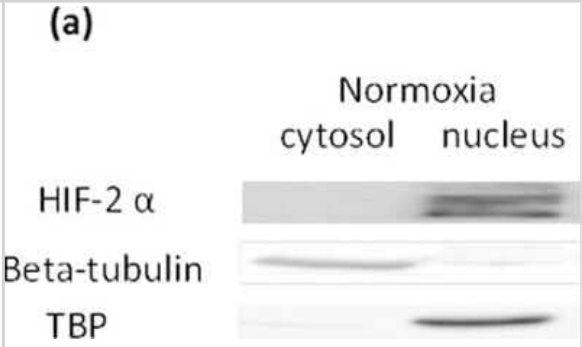
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	50 kDa

Product Description	
Host	Rabbit
Gene ID	203068
Gene Symbol	TUBB
Species	Human, Mouse, Rat, Porcine, Bovine, C. elegans, Chicken, Chinese Hamster, Insect, Invertebrate, Primate, Xenopus, Zebrafish
Reactivity Notes	Use in Rat reported in scientific literature (PMID:34519641). Invertebrate reactivity reported in scientific literature (PMID: 28114363). C. elegans reactivity reported in scientific literature (PMID: 27690361). Insect reactivity reported from a verified customer review.
Marker	Microtubule Marker
Immunogen	A synthetic peptide made to the N-terminal region of human beta Tubulin (within residues 1-100). [Swiss-Prot: P07437]

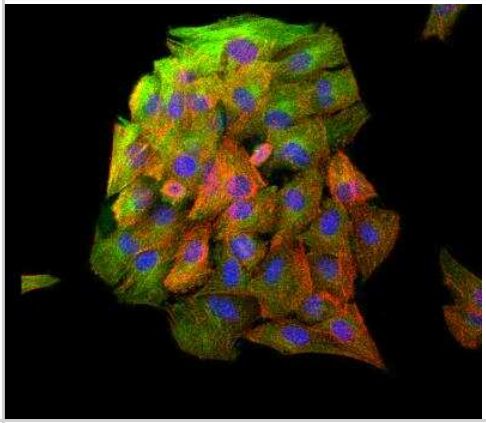
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:1000. Use reported in scientific literature (PMID 35008713), Immunohistochemistry 1:1000 - 1:2000, Immunocytochemistry/ Immunofluorescence 1:500 - 1:1000, Immunohistochemistry-Paraffin 1:1000 - 1:2000
Application Notes	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: separated by Size, antibody dilution of 1:500. Separated by Size-Wes, Sally Sue/Peggy Sue. SCW validated using murine pancreatic cancer cells.

Images

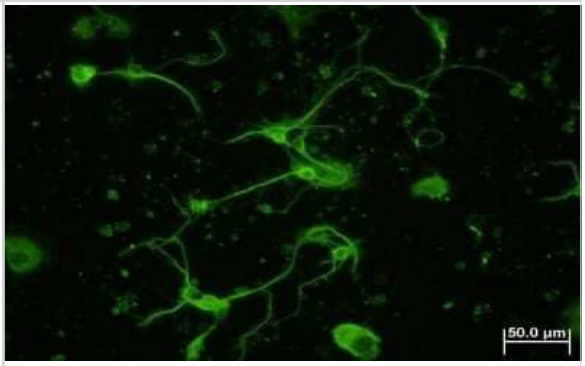
Immunocytochemistry/Immunofluorescence: beta Tubulin Antibody [NB600-936] - MCF7 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- (NB600-936) at 2 ug/ml overnight at 4C and detected with an anti-rabbit 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.	
Simple Western: beta Tubulin Antibody [NB600-936] - Lane view shows a specific band for beta Tubulin in 0.05 mg/ml of MCF-7 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: beta Tubulin Antibody [NB600-936] - Analysis of extracts from HeLa, A549 and Jurkat cells using NB600-936 Beta-tubulin antibody at 1:1000	
Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - beta Tubulin Antibody [NB600-936] - Reduced levels of NADPH oxidases 1 and 4 at 18% versus 5% O2. (a) Representative Western blots showing Nox1 and Beta-tubulin in PC-3 cells or Nox4 and Beta-tubulin in C2C12 cells, at 5% and 18% O2. (b, c) Average Nox1 signal How Supraphysiological Oxygen Levels in Standard Cell Culture Affect Oxygen-Consuming Reactions. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/30363917/) licensed under a CC-BY license.	

Immunocytochemistry/Immunofluorescence: beta Tubulin Antibody [NB600-936] - PC12 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-beta Tubulin Antibody (NB600-936) at 2 ug/ml overnight at 4C and detected with an anti-rabbit 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.	
Immunohistochemistry-Paraffin: beta Tubulin Antibody [NB600-936] - Analysis of FFPE tissue section of normal human skin using 1:2000 dilution of beta Tubulin antibody. Intense cytoplasmic staining of beta Tubulin (TUBB) protein was observed in various cells of the epidermal as well as the dermal cells [10X Magnification].	
Western Blot: beta Tubulin Antibody [NB600-936] - Analysis in HeLa whole cell lysate at a 1:1,000 dilution.	
Western Blot: beta Tubulin Antibody [NB600-936] - Expression of pluripotency related markers and a sphere culture of BEAS-2B under normoxia. HIF-2alpha expression was detected in the nucleus of BEAS-2B under normoxia by western blotting. Beta tubulin and TATA binding protein (TBP) were used as protein markers for the cytosol and nucleus fraction, respectively. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep29311), licensed under a CC-BY license.	

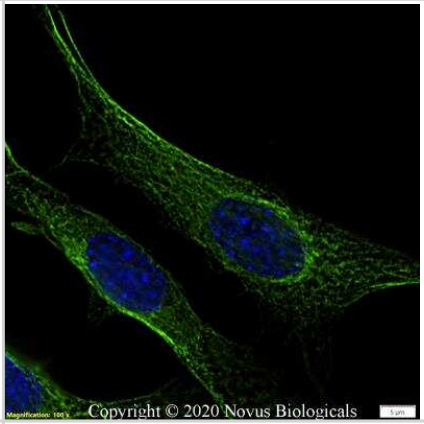
Immunocytochemistry/Immunofluorescence: beta Tubulin Antibody [NB600-936] - Confocal immunofluorescent analysis of C2C12 cells using beta Tubulin antibody (NB600-936, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).



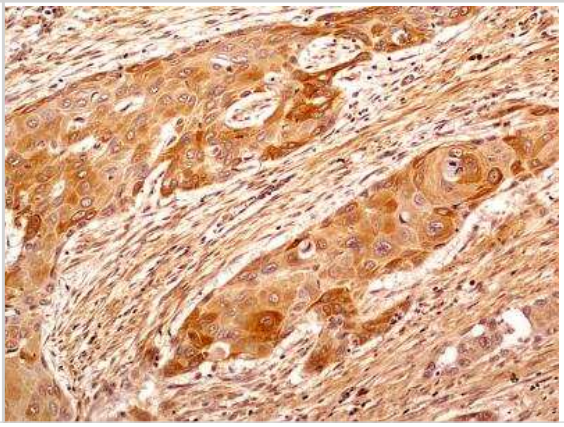
Immunocytochemistry/Immunofluorescence: beta Tubulin Antibody [NB600-936] - Analysis of beta Tubulin in mouse hippocampal primary culture. Image courtesy of product review by Lin Yi-Wen.

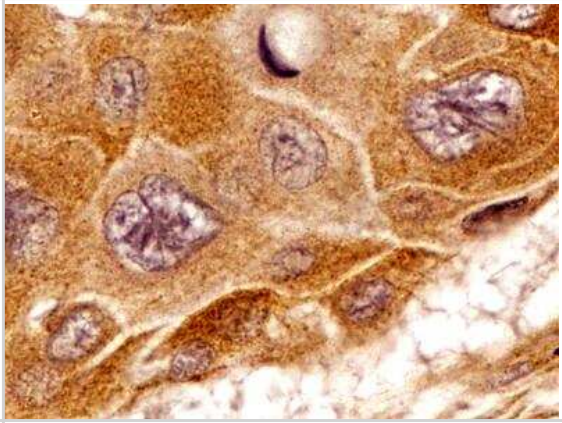
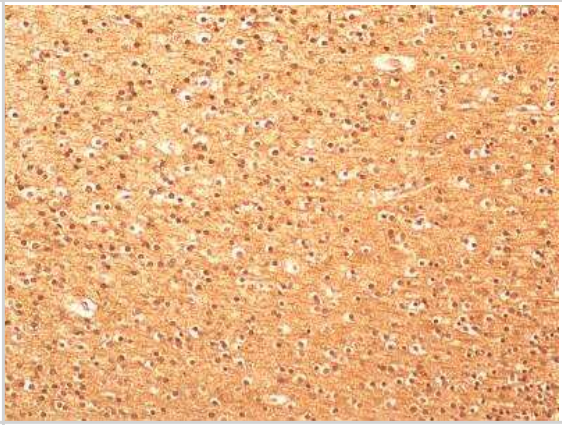
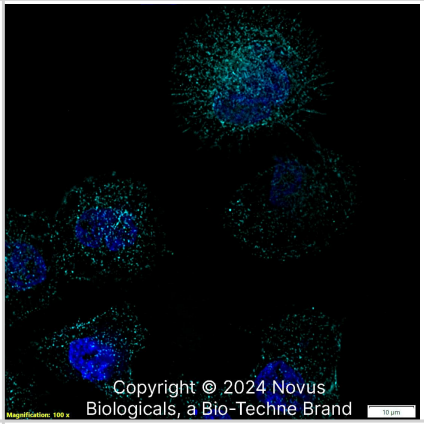


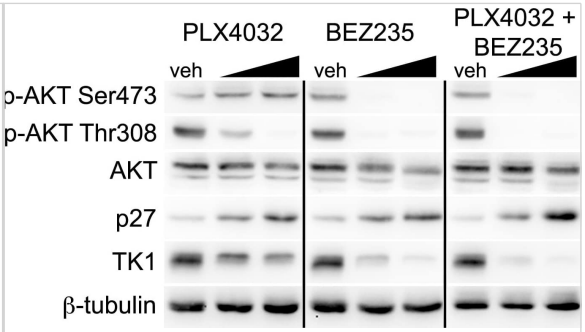
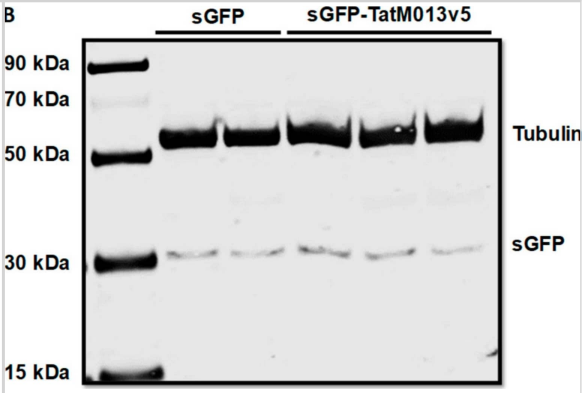
Immunocytochemistry/Immunofluorescence: beta Tubulin Antibody [NB600-936] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-beta Tubulin Antibody (NB600-936) at 2 ug/ml overnight at 4C and detected with an anti-rabbit 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.



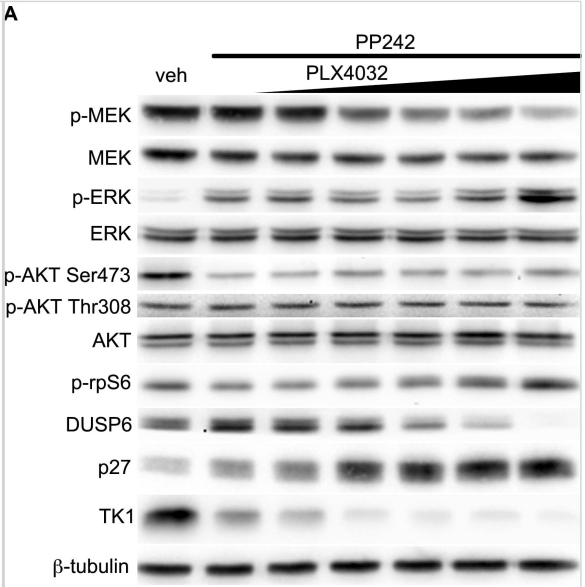
Immunohistochemistry-Paraffin: beta Tubulin Antibody [NB600-936] - Analysis of FFPE tissue section of human esophageal squamous cell carcinoma (SCC) using 1:2000 dilution of beta Tubulin antibody. Strong cytoplasmic immuno-positivity of beta Tubulin (TUBB) was observed in SCC cells as well as the associated tumor stromal cells [10X Magnification].



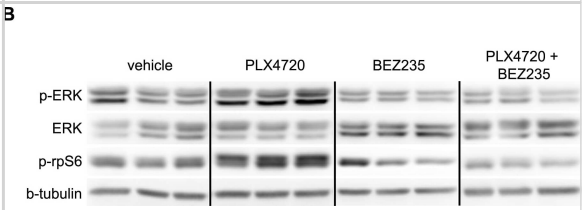
Immunohistochemistry-Paraffin: beta Tubulin Antibody [NB600-936] - Analysis of FFPE tissue section of human esophageal squamous cell carcinoma (SCC) using 1:2000 dilution of beta Tubulin antibody. This representative image shows a cytoplasm specific staining of beta Tubulin (TUBB) in SCC cells [60X Magnification].	
Immunohistochemistry-Paraffin: beta Tubulin Antibody [NB600-936] - Analysis of FFPE tissue section of normal human brain using 1:2000 dilution of beta Tubulin antibody. The various brain cells depicted strong cytoplasmic immunoreactivity of beta Tubulin (TUBB) protein [10X Magnification].	
Beta Tubulin was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti-beta Tubulin Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB600-936AF647) (light blue) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Beta Tubulin was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti-beta Tubulin Affinity Purified Polyclonal Antibody conjugated to DyLight 488 (Catalog # NB600-936G) (green) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.	

Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - Dual pathway inhibition regulates TK1 protein levels & results in greater p27 protein levels than single agents alone.Single agent PLX4032 resulted in activation of p-AKT Ser473 following 24 hours of exposure at two concentrations (100 nM, 1 µM). The addition of the dual PI3K/mTOR inhibitor BEZ235 blocks p-AKT Ser473 activation & resulted in a greater increase in p27 protein levels & diminished TK1 protein levels. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25247710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - Expression of pluripotency related markers & a sphere culture of BEAS-2B under normoxia.(a) HIF-2α expression was detected in the nucleus of BEAS-2B under normoxia by western blotting. Beta tubulin & TATA binding protein (TBP) were used as protein markers for the cytosol & nucleus fraction, respectively. (b) Western blotting for Oct-4. (c) For knockdown of HIF-2α, BEAS-2B cells were transfected with shRNA targeting HIF-2α & sh-Luc was used as control. (d) Western blotting of Nanog & a culture of BEAS-2B containing floating spheres. Representative result from two or three experiments was presented. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27373565), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - Expression of AAV transgene before & after experimental autoimmune uveoretinitis (EAU) induction. (A) B10.RIII mice were evaluated by funduscopy one month after an intravitreal injection of AAV delivering sGFP-TatM013v5 or secreted GFP (sGFP). As a control we evaluated mice that received an intravitreal injection of saline. Diffuse fluorescence indicates the secretion of sGFP & sGFP-TatM013v5 in the retina. (B) Western blot from retinas harvested 14 days after IRBP immunization. Membrane was probed with anti-GFP & anti-Tubulin antibodies. Image shows expression of sGFP on both sGFP & sGFP-TatM013v5 retina lysates. (n = 2–3 retina samples from different mice per group). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31795515), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

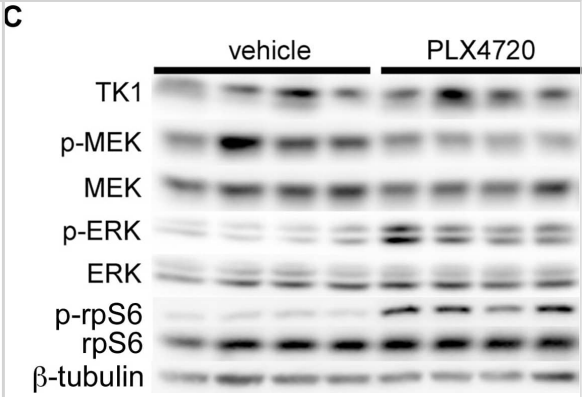
Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - Combined V600EBRAF & mTOR inhibition results in transcriptional control of TK1 protein levels in COLO 205 cells.(A) Western blot of COLO 205 cells treated with PP242 (250 nM) & increasing PLX4032. Similar to single agent PLX4032, p-MEK, but not p-ERK, was inhibited in a PLX4032-dependent manner. Consistent with mTORC1/mTORC2 inhibition, p-AKT Ser473, but not p-AKT Thr308, was inhibited. Unlike single agent PLX4032, which resulted in concentration-dependent activation of p-rpS6, combined treatment maintained p-rpS6 levels at essentially baseline levels except at the highest PLX4032 concentration. Similarly, DUSP6 levels were inversely related to p-ERK protein levels. With combined mTOR & V600EBRAF blockade, p27 & TK1 protein levels were inversely correlated & dramatically affected by PLX4032 exposure. (B) Similarly, TK1 mRNA was significantly reduced at PLX4032 concentrations as low as 10 nM. (C) Despite elevated p27 protein levels, p27 mRNA was unaffected by combined mTOR-V600EBRAF inhibition. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25247710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

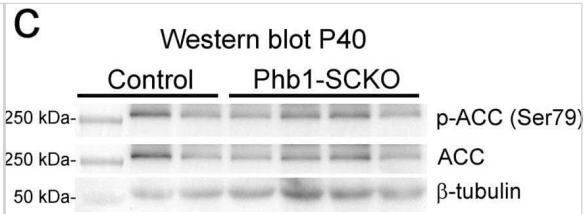
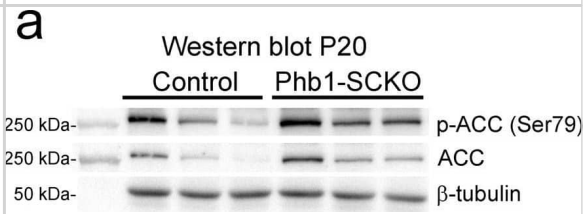


Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - [18F]-FLT PET reflects BEZ235-dependent inhibition of PI3K/mTOR activity in PLX4720 treated COLO 205 xenografts. Xenograft-bearing mice were imaged with [18F]-FLT PET on treatment day 4. (A) [18F]-FLT uptake was diminished in the combination treatment cohort relative to vehicle (p =0.0087), but not single agent PLX4720- or BEZ235-treated cohorts. (B) Western blot of xenograft tissue harvested immediately following imaging illustrated elevated p-ERK & p-rpS6 levels in PLX4720-treated mice. Combining PLX4032 with BEZ235 resulted in reduced p-ERK & p-rpS6 protein levels. (C) TK1 levels, as measured by IHC, were reduced only in the combination treatment group in agreement with [18F]-FLT PET. (D) Consistent with in vitro studies, diminished TK1 levels, & consequently [18F]-FLT PET, correlated with elevated p27 that was elevated only in the combination treated group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25247710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

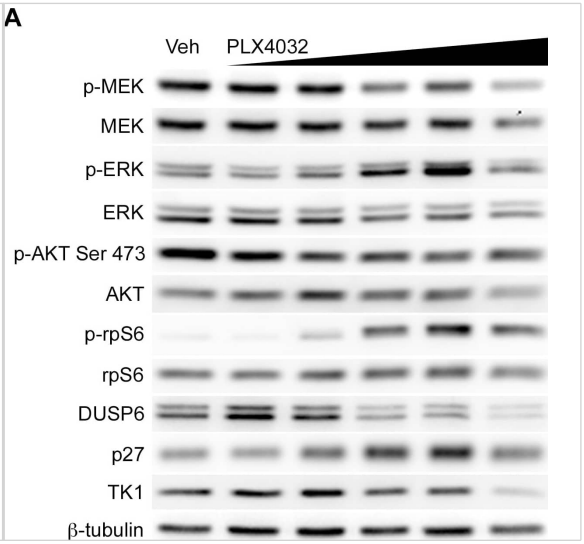


Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - PLX4720 exposure does not affect [18F]-FLT PET in COLO 205 xenografts, despite evidence of target inhibition & diminished [18F]-FDG uptake.(A) Representative transverse [18F]-FLT & [18F]-FDG PET images acquired after three daily treatments with vehicle or 60 mg/kg PLX4720 (tumor indicated by arrowhead). (B) Quantification of PET data illustrated similar [18F]-FLT uptake in vehicle-treated & PLX4720-treated tumors. Unlike [18F]-FLT PET, PLX4720 exposure elicited a significant reduction in [18F]-FDG uptake (p=0.0006). (C) Western blot analysis of vehicle- & PLX4720-treated tumor tissue confirmed that PLX4720 had no effect on TK1 protein levels in agreement with [18F]-FLT PET. Target inhibition as measured by p-MEK levels was observed. However, similar to in vitro studies, PLX4720-treated COLO 205 xenografts exhibited elevated p-ERK & p-rpS6 protein levels relative to vehicle controls. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25247710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - Deletion of Phb1 affects lipid metabolism.Western blot (a) & quantification (b) of Acetyl-CoA carboxylase (ACC) & phosphorylated ACC (p-ACC) expression at P20. N = 6–7 animals per genotype. Unpaired two-tailed t-test [p-ACC (t = 0.4627, df = 11, p = 0.021), ACC (t = 1.355, df = 11, p = 0.26)]. Western blot (c) & quantification (d) of ACC & p-ACC expression at P40. N = 6–8 animals per genotype [p-ACC (t = 0.5447, df = 12, p = 0.42), ACC (t = 1.153, df = 12, p = 0.17)]. Unpaired two-tailed t-test. By RT-qPCR, we identified a significant downregulation of many enzymes involved with lipid biosynthesis at both P20 (e) & P40 (f): sterol regulatory element-binding protein 1 (Srebp1), 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr), ATP citrate lyase (Acly), fatty acid synthase (FASN), acetyl-CoA carboxylase 2 (ACC2), N = 5 animals per genotype. Unpaired two-tailed t-test P20 [Srebp1 (t = 3.26, df = 8, p = 0.012), Hmgcr (t = 7.63, df = 8, p = 0.000061), Acly (t = 4.418, df = 8, 0.0022), FASN (t = 4.109, df = 8, p = 0.0034), ACC2 (t = 3.408, df = 8, p = 0.0092)]; P40 [Srebp1 (t = 7.551, df = 8, p = 0.000066), Hmgcr (t = 5.091, df = 8, p = 0.00094), Acly (t = 4.934, df = 8, p = 0.0011), FASN (t = 7.186, df = 8, p = 0.000094), ACC2 (t = 2.697, df = 8, p = 0.027)]. Data are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001. n.s. non-significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34078899), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - Deletion of Phb1 affects lipid metabolism.Western blot (a) & quantification (b) of Acetyl-CoA carboxylase (ACC) & phosphorylated ACC (p-ACC) expression at P20. N = 6–7 animals per genotype. Unpaired two-tailed t-test [p-ACC (t = 0.4627, df = 11, p = 0.021), ACC (t = 1.355, df = 11, p = 0.26)]. Western blot (c) & quantification (d) of ACC & p-ACC expression at P40. N = 6–8 animals per genotype [p-ACC (t = 0.5447, df = 12, p = 0.42), ACC (t = 1.153, df = 12, p = 0.17)]. Unpaired two-tailed t-test. By RT-qPCR, we identified a significant downregulation of many enzymes involved with lipid biosynthesis at both P20 (e) & P40 (f): sterol regulatory element-binding protein 1 (Srebp1), 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr), ATP citrate lyase (Acly), fatty acid synthase (FASN), acetyl-CoA carboxylase 2 (ACC2), N = 5 animals per genotype. Unpaired two-tailed t-test P20 [Srebp1 (t = 3.26, df = 8, p = 0.012), Hmgcr (t = 7.63, df = 8, p = 0.000061), Acly (t = 4.418, df = 8, 0.0022), FASN (t = 4.109, df = 8, p = 0.0034), ACC2 (t = 3.408, df = 8, p = 0.0092)]; P40 [Srebp1 (t = 7.551, df = 8, p = 0.000066), Hmgcr (t = 5.091, df = 8, p = 0.00094), Acly (t = 4.934, df = 8, p = 0.0011), FASN (t = 7.186, df = 8, p = 0.000094), ACC2 (t = 2.697, df = 8, p = 0.027)]. Data are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001. n.s. non-significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34078899), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: beta Tubulin Antibody - BSA Free [NB600-936] - TK1 protein levels do not reflect p-ERK attenuation following inhibition of V600EBRAF inhibition in COLO 205 cells.COLO 205 cells were collected 48 hours of PLX 4032 exposure at 10 nM, 100 nM, 500 nM, 1 µM, or 5 µM. (A) Western blot analysis demonstrated target inhibition of p-MEK despite increased p-ERK levels. PI3K-mTOR signaling was elevated in a PLX 4032-dependent manner as exhibited by a steady rise in p-rpS6 levels. The ERK-phosphatase DUSP6 decreased in conjunction with mTOR signaling & was inversely proportional to p-ERK levels. A slight increase in p27 levels were observed concomitantly with only modest changes in TK1 levels, except at the highest dose of PLX4032. (B) Decreased TK1 mRNA levels were observed at all drug concentrations above 10 nM ($p<0.05$). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25247710), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Longaretti A, Forastieri C, Gabaglio M et Al. Termination of acute stress response by the endocannabinoid system is regulated through lysine-specific demethylase 1-mediated transcriptional repression of 2-AG hydrolases ABHD6 and MAGL J. Neurochem. 2020-03-05 [PMID: 32141088]

Lu X, Ding F, Chen Y, Ke S et Al. Deficiency of C1QL1 Reduced Murine Ovarian Follicle Reserve Through Intraovarian and Endocrine Control Endocrinology 2022-05-13 [PMID: 35560215]

NI Weinstock, C Kreher, J Favret, D Nguyen, ER Bongarzone, L Wrabetz, M Laura Felt, D Shin Brainstem development requires galactosylceramidase and is critical for pathogenesis in a model of Krabbe disease Nat Commun, 2020-10-23;11(1):5356. 2020-10-23 [PMID: 33097716]

VerPlank JJ, Gawron JM, Silvestri NJ et Al. Knockout of PA200 improves proteasomal degradation and myelination in a proteotoxic neuropathy Life Sci Alliance 2024-02-06 [PMID: 38320810]

Wilson ER, Nunes GD, Shen S et Al. Loss of prohibitin 2 in Schwann cells dysregulates key transcription factors controlling developmental myelination Glia 2024-10-30 [PMID: 39215540]

Tan LX, Toops KA, Lakkaraju A. Protective responses to sublytic complement in the retinal pigment epithelium Proc. Natl. Acad. Sci. U.S.A. 2016-08-02 [PMID: 27432952]

Moore SM, Gawron J, Stevens M et al. Pharmacologically increasing cGMP improves proteostasis and reduces neuropathy in mouse models of CMT1 Cellular and Molecular Life Sciences: CMLS 2024-10-14 [PMID: 39400753]

Kai Zhao, Madita Braun, Leonie Meyer, Katharina Otte, Hartmann Raifer, Frederik Helmprobst, Vincent Möschl, Axel Pagenstecher, Hans Urban, Michael W. Ronellenfitsch, Joachim P. Steinbach, Jelena Pesek, Bernhard Watzer, Wolfgang A. Nockher, R. Verena Taudte, Andreas Neubauer, Christopher Nimsky, Jörg W. Bartsch, Tillmann Rusch, Swapan K. Ray A Novel Approach for Glioblastoma Treatment by Combining Apoptosis Inducers (TMZ, MTX, and Cytarabine) with E.V.A. (Eltanexor, Venetoclax, and A1210477) Inhibiting XPO1, Bcl-2, and Mcl-1 Cells 2024-04-04 [PMID: 38607071]

Hiral Shah, Marine Olivetta, Chandni Bhickta, Paolo Ronchi, Monika Trupinić, Eelco C Tromer, Iva M Tolić, Yannick Schwab, Omaya Dudin, Gautam Dey Life-cycle-coupled evolution of mitosis in close relatives of animals. Nature 2024-06-06 [PMID: 38778110]

Tessa Lord, Melissa J. Oatley, Jon M. Oatley Testicular Architecture Is Critical for Mediation of Retinoic Acid Responsiveness by Undifferentiated Spermatogonial Subtypes in the Mouse Stem Cell Reports 2018-02-01 [PMID: 29398482]

Andrew R. Castle, Sang-Gyun Kang, Ghazaleh Eskandari-Sedighi, Serene Wohlgemuth, My-Anh Nguyen, Daniel J. Drucker, Erin E. Mulvihill, David Westaway Beta-endoproteolysis of the cellular prion protein by dipeptidyl peptidase-4 and fibroblast activation protein Proceedings of the National Academy of Sciences of the United States of America 2022-12-27 [PMID: 36574660]

Neggers JE, Vanstreels E, Baloglu E et al. Heterozygous mutation of cysteine528 in XPO1 is sufficient for resistance to selective inhibitors of nuclear export Oncotarget 2016-10-18 [PMID: 27634897]

More publications at http://www.novusbio.com/NB600-936

Procedures

Western Blot protocol for beta Tubulin Antibody (NB600-936)

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 manufacturers instructions.

Immunocytochemistry/ Immunofluorescence Protocol for beta Tubulin Antibody (NB600-936)
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 beta Tubulin Antibody (NB600-936)
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 all the time).

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.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
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.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

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NB800-PC1	HeLa Whole Cell Lysate
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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
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