

Product Datasheet

Ki67/MKI67 Antibody - BSA Free NB110-89717

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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NB110-89717

Ki67/MKI67 Antibody - BSA Free

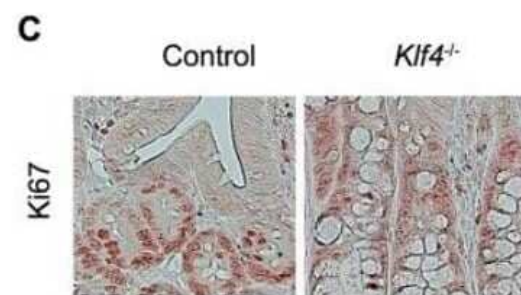
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	351 kDa
Product Description	
Description	Novus Biologicals Knockout (KO) Validated Rabbit Ki67/MKI67 Antibody - BSA Free (NB110-89717) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-Ki67/MKI67 Antibody: Cited in 91 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	4288
Gene Symbol	MKI67
Species	Human, Mouse, Rat, Porcine
Reactivity Notes	Ki67/MKI67 Antibody reacted with Rat in in scientific literature (PMID: 24275061). Use in Porcine reported in scientific literature (PMID:32132871).
Marker	Proliferation Marker
Immunogen	The immunogen for this KI67/MKI67 Antibody was made using a synthetic peptide from the internal region of Mouse KI67/MKI67, between aminoacids 1850-1950 (1899-1916) Uniprot# E9PVX6.
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Knockout Validated
Recommended Dilutions	Western Blot 1:100-1:2000. Use reported in scientific literature (PMID 22384261), Flow Cytometry 1:100. Use reported by customer review, Immunohistochemistry reported in scientific literature (PMID 28832561), Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 24779589), Immunohistochemistry-Paraffin 1:100-1:500, Immunohistochemistry-Frozen, Flow (Intracellular), Knockout Validated

Images

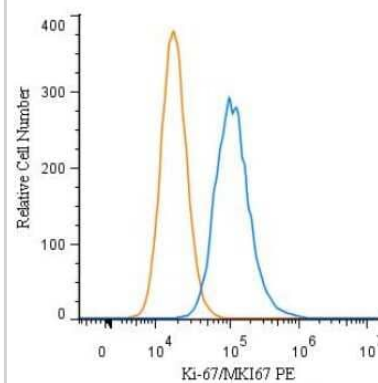
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NB110-89717 at 2 ug/ml overnight at 4C and detected with 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.



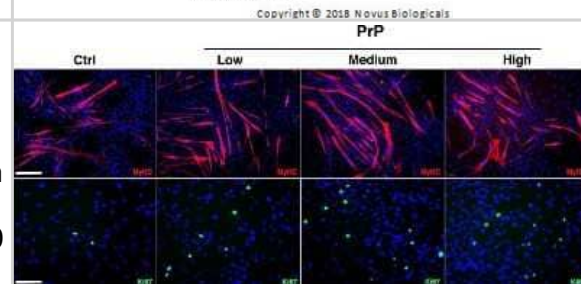
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - KLF4 ablation leads to abnormal proliferation and differentiation in small intestinal epithelium. Higher magnification IHC staining of highlighted frames. Small intestine from *Klf4*^{-/-} mice induced by tamoxifen for different time endurances were stained by H&E and PAS, and also immunohistochemistry staining was performed with anti-Ki67, anti-Lysozyme, anti-DCAMKL-1, and anti-PCNA antibodies respectively. Bottom panel: IHC staining with ZO-1 antibody in one-month knockout intestine tissue. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0032492>) licensed under a CC-BY license.



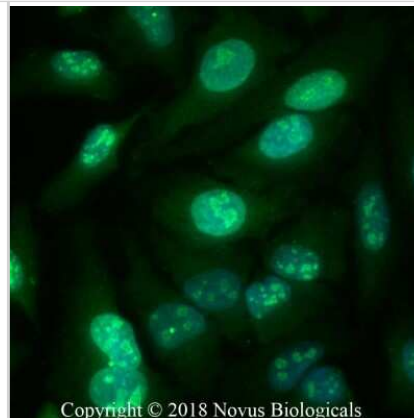
Flow Cytometry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - An intracellular stain was performed on HeLa cells with Ki-67/MKI67 antibody NB110-89717PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.



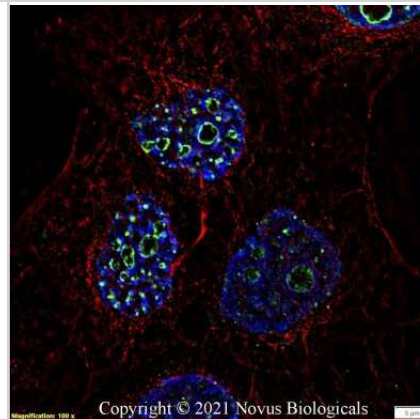
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Immunofluorescences against MyHC (red) and Ki67/MKI67 (green) revealing differences in proliferation and differentiation in relation to different PrP concentration. alpha-MEM supplemented with: Ctrl (20% FBS), Low (5×10^5 platelets/ml), Medium (1×10^6 platelets/ml), and High (1.5×10^6 platelets/ml); nuclei were counterstained in blue by DAPI. Scale bars: MyHC = 200 um; Ki67 = 100 um. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/33041857/>) licensed under a CC-BY license.



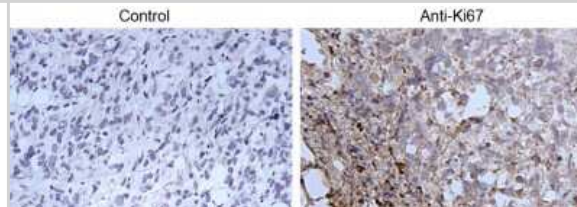
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-Ki-67/MKI67 at 2 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (green) at a 1:500 dilution. Nuclei were counterstained with DAPI (blue). Cells were imaged using a 40X objective.



Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Ki67/MKI67 Antibody NB110-89717 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. 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 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



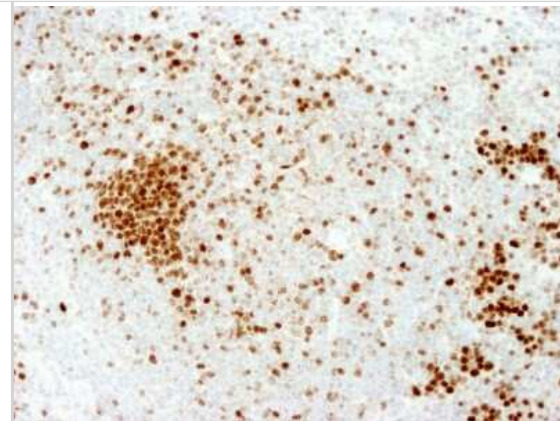
Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Analysis of Ki-67 in paraffin embedded mouse prostate tissue using anti-Ki-67 antibody. Image from verified customer review.



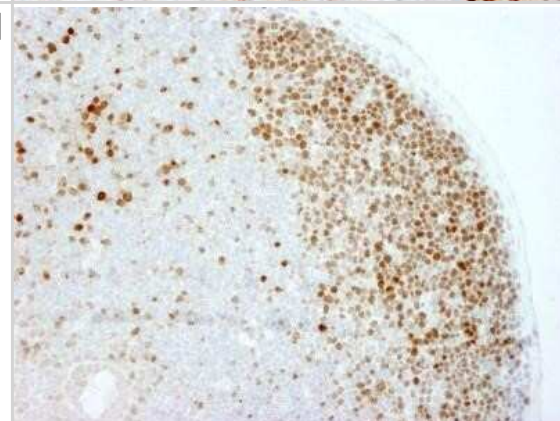
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Ki-67/MKI67 Antibody [NB110-89717] - Analysis of a mouse intestine cross section. The antibody was used at a dilution of 1:250. Detection: DAB staining. Epitope Retrieval Buffer-High pH was substituted for Epitope Retrieval Buffer-Reduced pH.



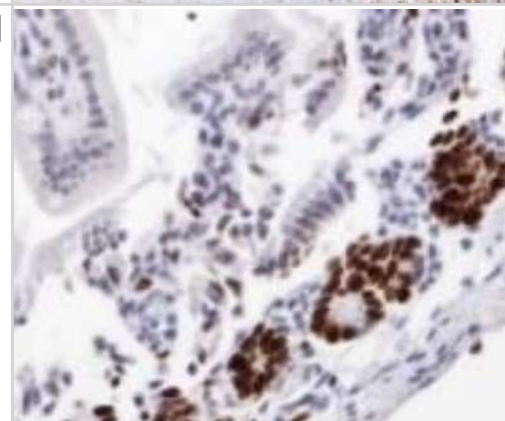
Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Staining of a cross section of mouse spleen. Detection: DAB staining using Immunohistochemistry Accessory Kit. Epitope Retrieval Buffer-High pH was substituted for Epitope Retrieval Buffer-Reduced pH.



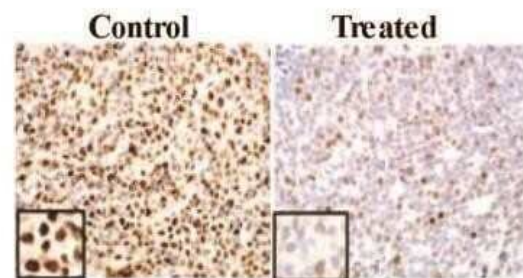
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Ki67 Antibody [NB110-89717] - FFPE section of mouse Peyer's patch. Antibody: Affinity purified rabbit anti-mouse Ki-67 used at a dilution of 1:250. Detection: DAB staining using Immunohistochemistry Accessory Kit. Epitope Retrieval Buffer-High pH was substituted for Epitope Retrieval Buffer-Reduced pH.



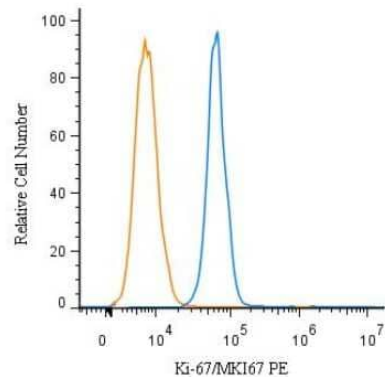
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Ki-67/MKI67 Antibody [NB110-89717] - Detection of Ki67 in FFPE mouse intestine using NB110-89717.



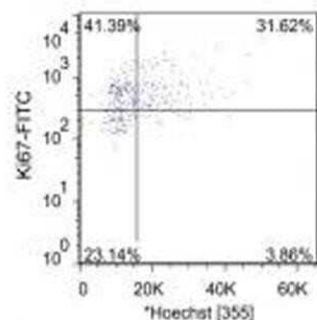
Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Analysis of Ki-67 in human prostate xenograft control (left) and treated (right) using anti-Ki-67 antibody. Image from verified customer review.



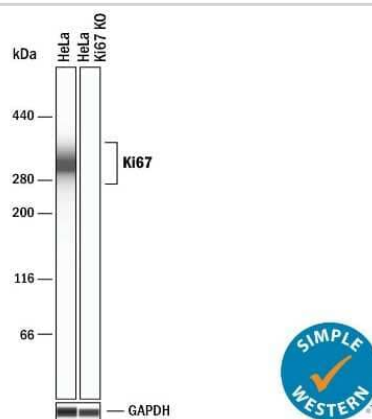
Flow Cytometry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - An intracellular stain was performed on U-937 cells with NB110-89717PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.



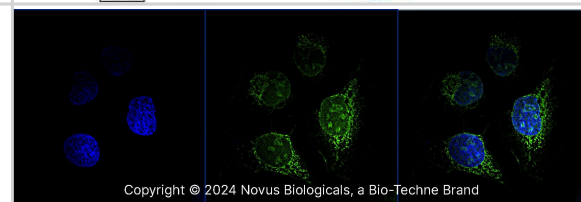
Flow Cytometry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Ki-67/MKI67 Antibody [NB110-89717] - Staining of mouse bone marrow cells using NB110-89717 at a dilution of 1:100. Photo courtesy of product review by verified customer.



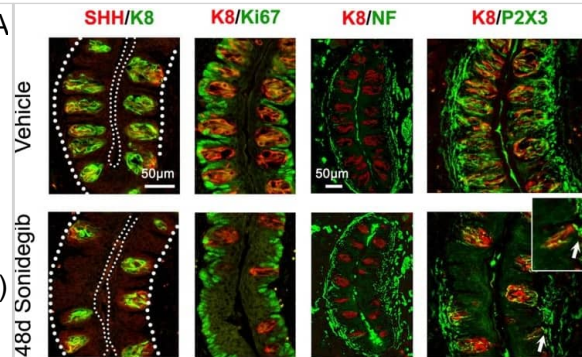
Simple Western: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Detection of Ki67/MKI67 by Simple Western™. Simple Western lane view shows lysates of HeLa parental cell line and Ki67 knockout (KO) HeLa cell line. A specific band was detected for Ki67/MKI67 at approximately 312 kDa (as indicated) in the parental cell line, but is not detectable in the knockout HeLa cell line using 20 ug/mL of Rabbit Anti-Ki67/MKI67 Polyclonal Antibody (Catalog # NB110-89717). GAPDH is shown as a loading control. This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



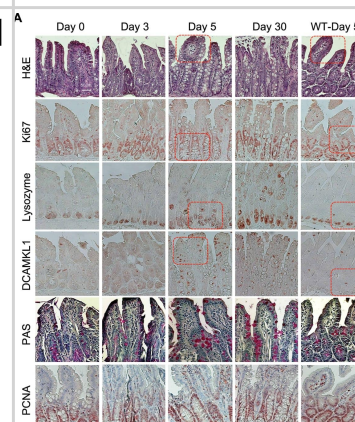
Ki67/MKI67 was detected in immersion fixed U-2 OS human osteosarcoma cell line using Rabbit anti- Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NB110-89717AF488) (green) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



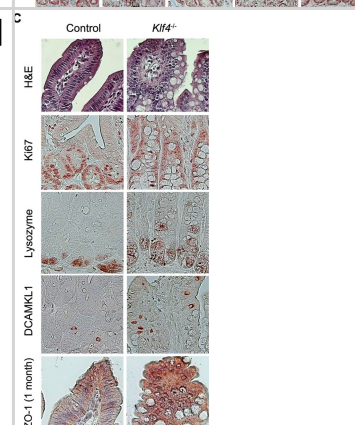
Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Long term Sonidegib treatment in mouse reduces taste buds (TB) & SHH ligand in circumvallate papilla (CV) whereas proliferation & innervation are retained. Immunofluorescent antibody detection of SHH ligand (red) & K8 (green) for TB cells; K8 (red) for TB cells & Ki67 (green) for cell proliferation; & K8 (red) with NF (green) for GL innervation or P2X3 (green) for GL taste fibers, after Vehicle or 48d Sonidegib treatment. For SHH/K8, large dotted lines indicate the basal lamina. Small dotted lines outline the surface epithelium. Inset (K8/P2X3) shows an image of nerves extending into CV epithelial basal lamina (arrow). Scale bar: 50 μ m, applies to all images. Inset at 2 \times . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30385780>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



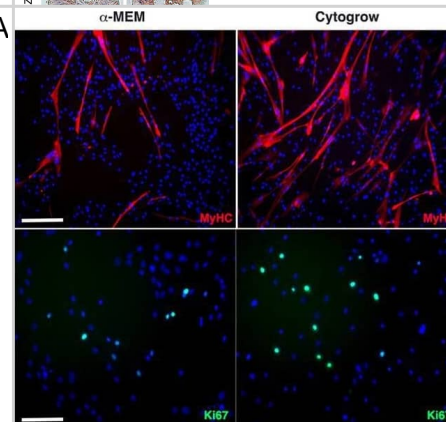
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - KLF4 ablation leads to abnormal proliferation & differentiation in small intestinal epithelium. (A) Small intestine from *Klf4*^{-/-} mice induced by tamoxifen for different time durations were stained by H&E & PAS, & also immunohistochemistry staining was performed with anti-Ki67, anti-Lysozyme, anti-DCAMKL-1, & anti-PCNA antibodies respectively. (B) Statistic analysis of IHC staining results from (A). (*, $P < 0.05$) (C) IHC staining from (A) in higher magnification of highlighted frames. Bottom panel: IHC staining with ZO-1 antibody in one-month knockout intestine tissue. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22384261>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



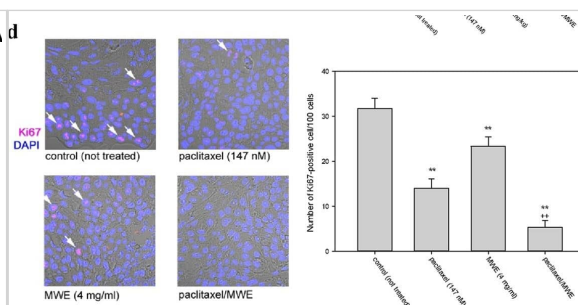
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - KLF4 ablation leads to abnormal proliferation & differentiation in small intestinal epithelium. (A) Small intestine from *Klf4*^{-/-} mice induced by tamoxifen for different time durations were stained by H&E & PAS, & also immunohistochemistry staining was performed with anti-Ki67, anti-Lysozyme, anti-DCAMKL-1, & anti-PCNA antibodies respectively. (B) Statistic analysis of IHC staining results from (A). (*, $P < 0.05$) (C) IHC staining from (A) in higher magnification of highlighted frames. Bottom panel: IHC staining with ZO-1 antibody in one-month knockout intestine tissue. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22384261>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



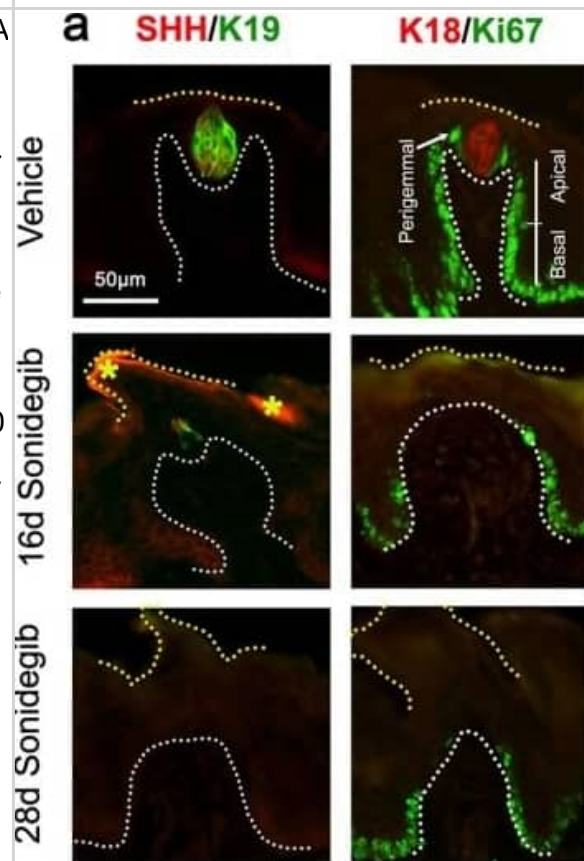
Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Culture conditions influence cell behavior. Immunofluorescences against MyHC (red) & Ki67 (green) reveal the remarkable differences in terms of differentiation & proliferation employing Cyto-Grow commercial medium compared with α -MEM (nuclei labeled in blue by DAPI). The respective quantifications are reported as fusion index & rate of proliferating nuclei (Ki67 positive). Statistical analysis: one-way ANOVA & Tukey's test. ** $p < 0.01$, *** $p < 0.001$ ($n = 3$). Scale bars: MyHC = 200 μ m; Ki67 = 100 μ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/33041857>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



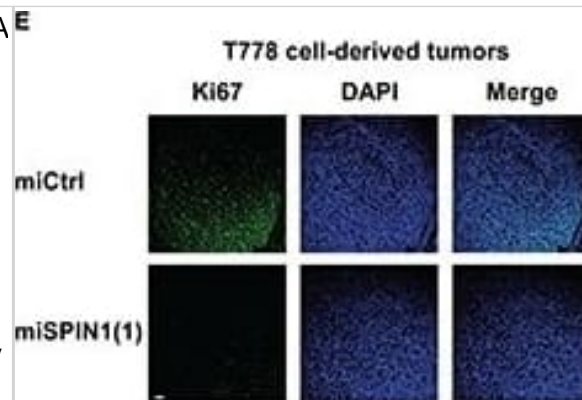
Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Paclitaxel in combination with MWE retarded tumor growth in a human bladder carcinoma TSGH 8301 xenograft model. (a) TSGH 8301 cells (1×10^7 cells/mouse) were injected into the right inguinal region of a nude mouse to form tumor xenografts. When the tumor size reached approximately 250 to 300 mm³, the mice were randomly divided into 4 groups & received the following treatments: paclitaxel combined with MWE, MWE alone, paclitaxel alone & sterile deionized water (control group). Tumor size was monitored every week, & the results are expressed as the percentage of the size at week 0 (the day treatment started) for each group. (b) The levels of total (t-PTEN) & phospho-PTEN (p-PTEN) & Caspase 3 in the tumor specimens were determined by Western blotting & then quantified using β -actin as the protein loading control; the results are expressed as a percentage of the control. (c) Immunohistochemical examination of p-PTEN in the tumor sections obtained from the indicated treatment. (d) & (e) Fluorescent immunohistochemical detection of Ki67 & TUNEL examination in the xenografts obtained from the indicated treatment. (f) Western blotting analysis of the levels of Cyclin B1, Cdc2 & Aurora A in xenograft tumors. Arrow indicates the Ki67 or TUNEL positive cells. One-way ANOVA with post-hoc Dunnett's test was used to calculate the p value for each treatment compared to paclitaxel alone, (+p < 0.05; ++p < 0.01) at each time point (**indicates p < 0.01 & *indicates p < 0.05). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep20417>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



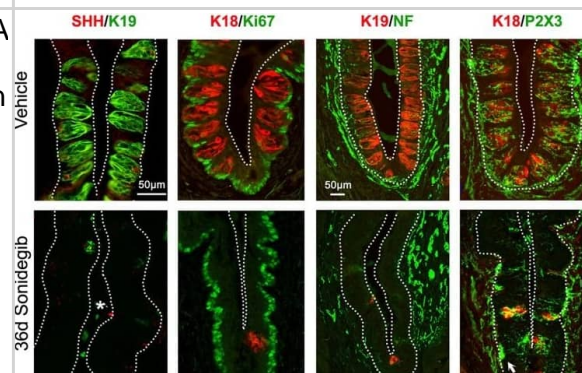
Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Sonidegib treatment reduces taste buds (TB), SHH ligand & proliferation in rat fungiform papilla (FP) while innervation is retained. (a) Immunofluorescent antibody detection of SHH ligand (red) & K19 (green) for TB cells; K18 (red) for TB cells & Ki67 (green) for cell proliferation, after Vehicle, 16d or 28d Sonidegib treatments. SHH is reduced in association with TB, K19+ cell loss. Asterisks (*) indicate nonspecific SHH immunoprodut in cornified surface cells in 16d Sonidegib image. The Vehicle, K18/Ki67 image shows 3 regions positive for Ki67+ cells (Apical, Basal & Perigemmal). Proliferating cells are lost in Apical FP region after 16–28d Sonidegib. (b) Number of Ki67+ cells in Apical & Basal regions of FP in Vehicle- & Sonidegib-treated mice. Numbers of tongues analyzed are in parentheses. For each tongue 8–10 FP were analyzed. Sonidegib treatment reduces apical epithelial cell proliferation in FP compared to Vehicle. Statistical analysis was one-way ANOVA with Tukey HSD posthoc comparisons (*p ≤ 0.05, compared to Vehicle, APICAL). (c) Immunofluorescent antibody detection of K19 or K18 (red) for TB cells & NF (green) for lingual & CT innervation or P2X3 (green) for CT nerve fibers. Innervation was retained after Sonidegib exposure. Asterisks (*) indicate nonspecific P2X3 immunoprodut in surface layer in Vehicle image. (d) Enlarged images from 28d Sonidegib papillae. Arrows point to NF+ or P2X3+ fibers in the FP epithelium. Throughout, white dotted lines indicate the basal lamina. Yellow dotted lines indicate surface of epithelium. (a,c) Scale bar: 50 μ m, applies to all images. (d) Scale bar: 25 μ m, applies to both images. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30385780>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



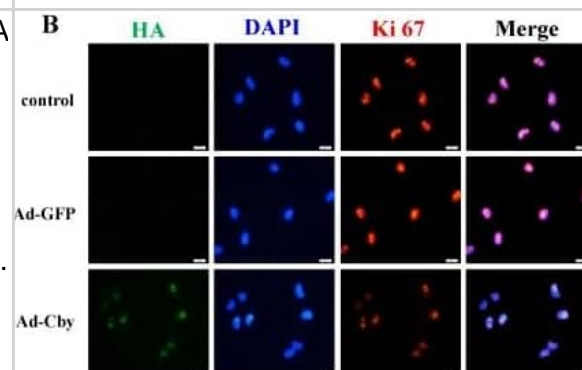
Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - SPIN1 controls proliferation & apoptosis of liposarcoma cell-derived tumors in BALB/c nude mice (A) Analysis of tumors from BALB/c nude mice (n = 15) 10 days after subcutaneous injection of T778 cells expressing control miRNA (miCtrl) or miRNA against SPIN1 [miSPIN1(1)]. Scale bar = 5 mm. (B) Average tumor weight of mice shown in (A) (C) Quantitative RT-PCR analysis of SPIN1, GDNF, & RET expression in T778 cell-derived tumors treated with the indicated miRNA. (D) Western blot analysis of SPIN1, RET, & RETph levels in T778 cell-derived tumors treated with the indicated miRNA. α -Tubulin & GFP were used as loading controls. (E) Detection of Ki67 by immunofluorescence in T778 cell-derived tumors treated with the indicated miRNA. Scale bar = 100 μ m. (F) Quantification of Ki67 staining shown in (E). (G) TUNEL assay for detection of apoptotic cells in T778 cell-derived tumors treated with the indicated miRNA. Scale bar = 100 μ m. (H) Quantification of TUNEL staining shown in (G) (B, C, F, H) Error bars represent \pm SEM, * p < 0.05, ** p < 0.01. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/25749382>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



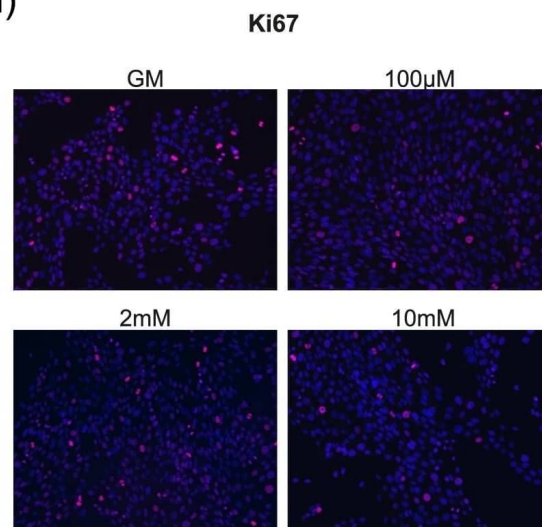
Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Sonidegib treatment in rat reduces taste buds (TB) & SHH ligand in circumvallate papilla (CV) whereas cell proliferation & GL innervation are retained. Immunofluorescent antibody detection of SHH ligand (red) & K19 (green) for TB cells; K18 (red) for TB cells & Ki67 (green) for cell proliferation; K19 (red) for TB cells & NF (green) for innervation; and, K18 (red) for TB cells & P2X3 (green) for taste nerve fibers, after Vehicle or 36d Sonidegib treatment. White dotted lines outline the epithelium. Asterisk in SHH/K19, 36d Sonidegib indicates nonspecific K19 immunostaining. Arrow points to the P2X3+ nerves extending into CV epithelium after Sonidegib treatment. SHH is reduced in association with TB cells. Cell proliferation is maintained & nerves fibers are retained after Sonidegib treatment. Scale bar: 50 μ m, applies to all images, except K19/NF. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30385780>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - Overexpression of Chibby expression inhibits cell proliferation & invasion of HCC cells. (A) Adenovirus-mediated ectopic expression of Chibby & enhanced the expression of Chibby in Huh7 cells. After 48 h of infection with adenoviral vectors (Ad-GFP or Ad-Chibby) at different multiplicity of infection, the protein lysates from the Huh7 cells were harvested to determine the ectopic gene expression using Western blot analysis. Ad-GFP was designed as the control vector. (B) Representative immunofluorescent images of Ki67 in Huh7-normal control, Huh7-Ad-GFP, & Huh7-Ad-Chibby cells. (C,D) The enhancement of Chibby in Huh7 cells transfected with Ad-Chibby suppressed cell proliferation & invasiveness by colon-formation assay & the Boyden chamber system, respectively. Data represent mean \pm SE from three independent analyses. Scale bar, 100 μ m, * p < 0.05 & ** p < 0.01 vs. Ad-GFP group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32192213>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB110-89717] - High doses of metformin inhibit the proliferation of C2C12 cells without inducing apoptosis. (a) C2C12 cells were treated with different doses of metformin in growth medium (GM) & the total number of cells was counted after 1, 2, 3 & 4 days of treatment by immunofluorescence microscopy. The initial number of plated cells was the same in each growth condition. Statistical significance was evaluated by the Student's t-test ($*p < 0.05$) (b) TUNEL assay. C2C12 cells were treated with 100 μ M, 2mM & 10mM metformin for 48h. As positive control for the TUNEL assay C2C12 myoblasts were incubated with DNase I before staining and, as negative control, cells were stained with the label solution without the addition of the reaction enzyme terminal deoxynucleotidyl transferase (TdT). (c) Total protein extracts of C2C12 myoblasts treated with 100 μ M, 2mM & 10mM were analyzed by SDS-PAGE for the expression of the apoptotic markers cl-caspase 3 & cl-caspase 7. For the induction of apoptosis in the positive control was used staurosporine 1 μ M for 4 hours. GAPDH is used as loading control (d) Proliferating C2C12 myoblasts, were plated at the same initial number (4×10^4 in GM in 9,5 cm² area wells), incubated with 100 μ M, 2mM & 10mM metformin for 48h. The percentage of cells expressing Ki67 was measured by Cell Profiler cell image analysis software. Statistical significance was evaluated by the Student's t-test ($*p < 0.05$). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28859084>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Ki67/MKI67 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti-Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to Janelia Fluor® 646 (Catalog # NB110-89717JF646) (light blue) at 5 μ g/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Steven A, Leisz S, Sychra K et al. Hypoxia-mediated alterations and their role in the HER-2/neuregulated CREB status and localization *Oncotarget* 2016-08-09 [PMID: 27409833] (Immunohistochemistry, Mouse)

Mouillet JF, Goff J, Sadovsky E et al. Transgenic expression of human C19MC miRNAs impacts placental morphogenesis *Placenta* 2020-11-01 [PMID: 33017713] (Immunohistochemistry, Mouse)

Fein MR, He XY, Almeida AS et al. Cancer cell CCR2 orchestrates suppression of the adaptive immune response *Journal of Experimental Medicine* 2020-10-05 [PMID: 32667673] (Immunohistochemistry, Mouse)

Burke-Kleinman J, Rubianto J, Hou G et al. Matrix-Binding, N-Cadherin-Targeting Chimeric Peptide Inhibits Intimal Thickening but Not Endothelial Repair in Balloon-Injured Carotid Arteries *Arteriosclerosis, Thrombosis, and Vascular Biology* 2023-09-01 [PMID: 37409527] (Immunohistochemistry, Mouse)

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Procedures

Immunohistochemistry Protocol specific for Ki67 Antibody (NB110-89717)

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.

Staining:

- 1) Wash sections in dH₂O three times for 5 minutes each.
- 2) Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
- 3) Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
- 4) Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
- 5) Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
- 6) Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.
- 7) Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
- 8) Add 100-400 ul Striavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
- 9) Wash sections three times in wash buffer for 5 minutes each.
- 10) Add 100-400 ul DAB substrate to each section and monitor staining closely.
- 11) As soon as the sections develop, immerse slides in dH₂O.
- 12) Counterstain sections in hematoxylin.
- 13) Wash sections in dH₂O two times for 5 minutes each.
- 14) Dehydrate sections.
- 15) Mount coverslips.

Immunocytochemistry/ Immunofluorescence Protocol for Ki67/MKI67 Antibody (NB110-89717)

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Flow (Intracellular) Protocol for Ki67/MKI67 Antibody (NB110-89717)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 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 μ L 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 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 μ L 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 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 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 μ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 μ g 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 μ L 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 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.



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