Product Datasheet

Ki67/MKI67 Antibody - BSA Free
NB500-170

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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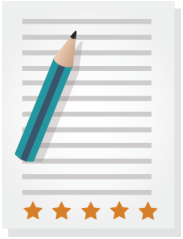
Updated 4/13/2025 v.20.1



NB500-170

Ki67/MKI67 Antibody - BSA Free

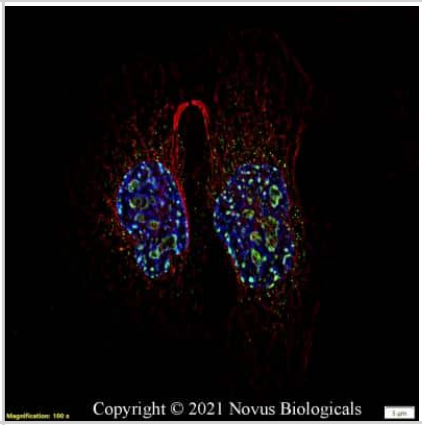
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	359 kDa

Product Description	
Host	Rabbit
Gene ID	4288
Gene Symbol	MKI67
Species	Human, Mouse, Rat, Porcine, Avian
Reactivity Notes	Use in Avian reported in scientific literature (PMID:34986451) Ki67/MKI67 Antibody reacted with Mouse (PMID: 22033079), Rat (PMID: 22521325), and Porcine (PMID: 29146772). .
Marker	Proliferation Marker
Immunogen	The immunogen for this KI67/MKI67 Antibody was made using a synthetic peptide from the internal region of Human KI67/MKI67, between amino acids: 1550-1600 [Uniprot: P46013].

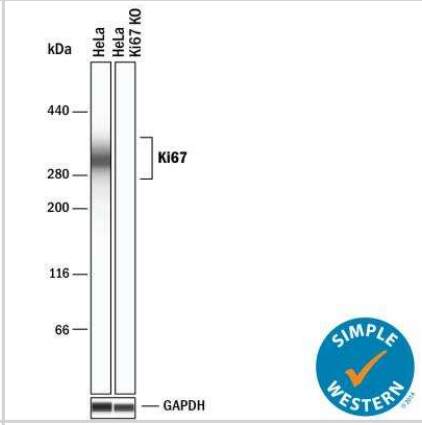
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot 1-2 ug/ml, Flow Cytometry reported in scientific literature (PMID 34946963), Immunohistochemistry 1:50-1:300, Immunocytochemistry/ Immunofluorescence 1:20-1:100, Immunoprecipitation reported by customer review, Immunohistochemistry-Paraffin 1:50-1:300, Immunohistochemistry-Frozen 1:50-1:300. Use reported in scientific literature (PMID 22493503), Immunoblotting reported in scientific literature (PMID 24248265), Knockout Validated
Application Notes	Formalin fixed paraffin embedded tissue sections require high temperature antigen unmasking with 10 mM citrate buffer (pH 6.0) prior to immunostaining. This antibody will not work without optimal antigen retrieval, and is a critical step. NOTE: We suggest an incubation period of 30 minutes at room temperature and to use DAB to stain the protein.

Images

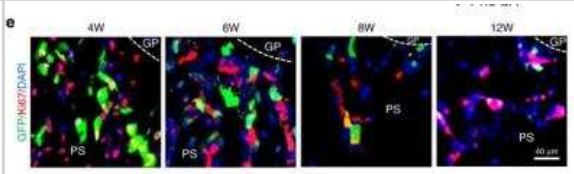
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody [NB500-170] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NB500-170 at 2 ug/ml overnight at 4C and detected with an anti-rabbit 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.



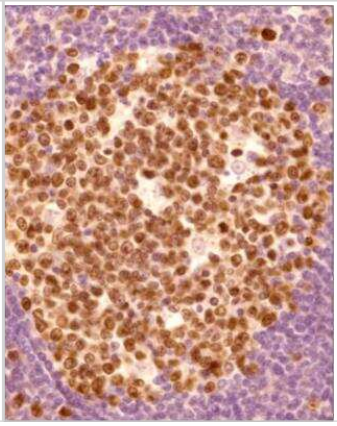
Simple Western: Ki67/MKI67 Antibody [NB500-170] - Detection of Ki67/MKI67 by Simple WesternTM. Simple Western lane view shows lysates of HeLa parental cell line and Ki67 knockout (KO) HeLa cell line. A specific band was detected for Ki67/MKI67 at approximately 320 kDa (as indicated) in the parental cell line, but is not detectable in the knockout HeLa cell line using 20 ug/mL of Rabbit Anti-Ki67/MKI67 Polyclonal Antibody (Catalog # NB500-170). GAPDH is shown as a loading control. This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



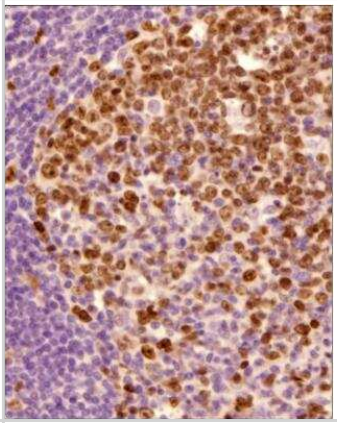
Immunohistochemistry: Ki67/MKI67 Antibody [NB500-170] - Senescent MSPCs are characterized by loss of nestin expression. Double-immunofluorescence images of femoral metaphysis sections from 4-, 6-, 8-, and 12-week-old male Nestin-GFP mice using antibodies against GFP (green) and Ki67 (red). DAPI stains nuclei blue. GP growth plate. PS primary spongiosa. Quantification of the percentage of GFP+ cells that express Ki67. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-017-01509-0) licensed under a CC-BY license.



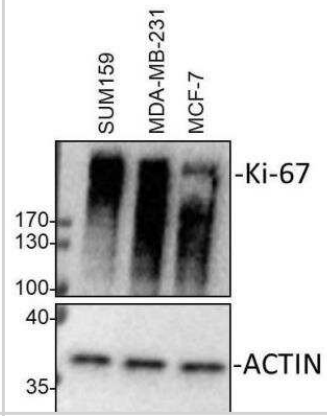
Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody [NB500-170] - Ki-67/MKI67 Antibody [NB500-170] - Tissue section of human tonsil using 1:50 dilution of rabbit anti-KI67 antibody. The staining was developed with HRP labeled anti-rabbit IgG secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This Ki67 antibody generated a specific nuclear staining in the cells in germinal centers of the tested tonsil tissue.



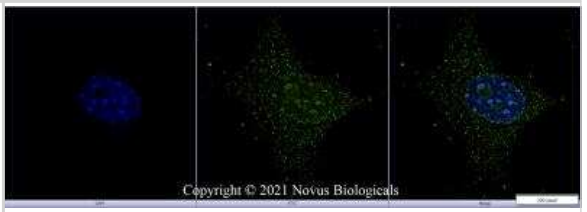
Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody [NB500-170] - Ki-67/MKI67 Antibody [NB500-170] - Human tonsil using 1:200 dilution of rabbit anti-KI67 antibody. The staining was developed with HRP labeled anti-rabbit IgG secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This Ki67 antibody generated a specific nuclear staining in the cells in germinal centers of the tested tonsil tissue.



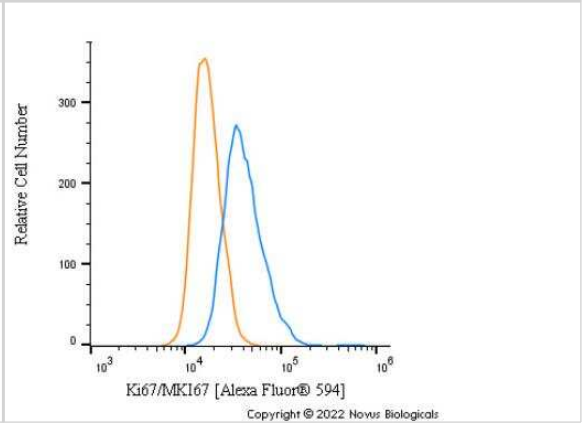
Western Blot: Ki67/MKI67 Antibody - BSA Free [NB500-170] - Whole cell lysates from SUM159, MDA-MB-231 and MCF-7 cells were loaded with 30 ug/lane. 10% SDS-PAGE. Ki67/MKI67 antibody (NB500-170) was used for primary antibody: 1:2000, 4C, overnight. Image from verified customer review.

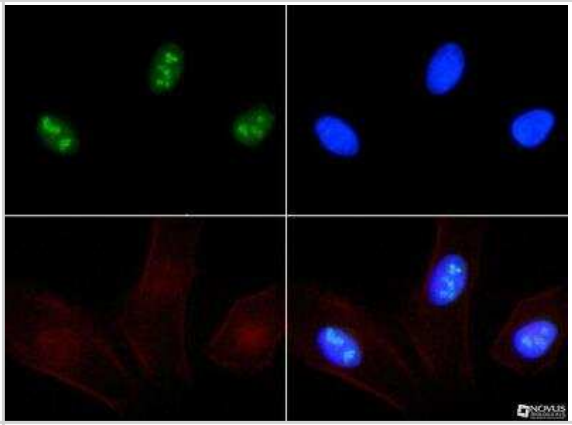
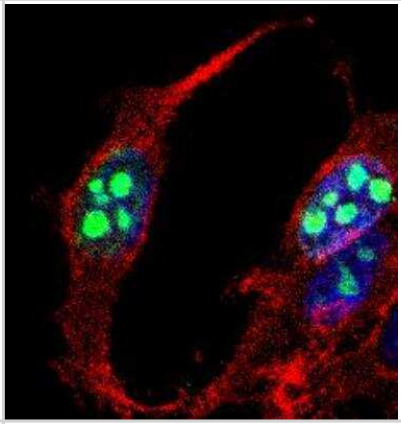
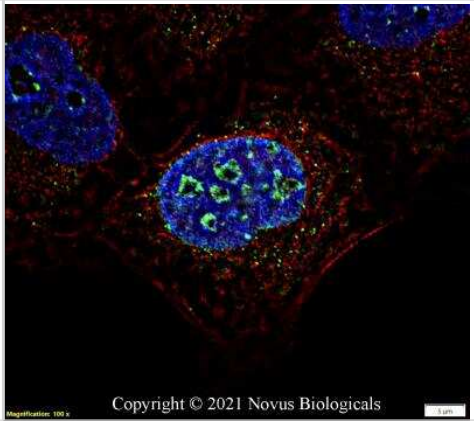


Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody [NB500-170] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Ki67/MKI67 Antibody NB500-170 at 2 ug/ml overnight at 4C and detected 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.

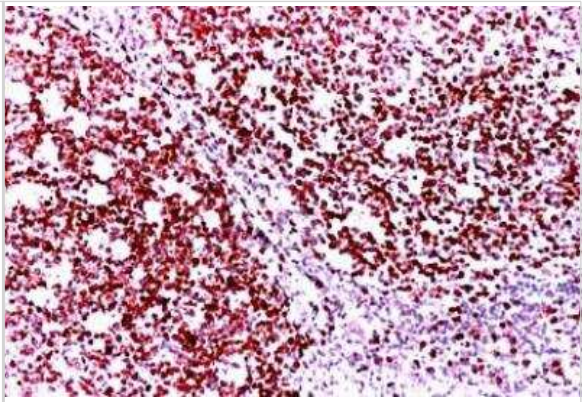


Flow Cytometry: Ki67/MKI67 Antibody - BSA Free [NB500-170] - An intracellular stain was performed on U-251 MG cells with Ki67/MKI67 Antibody NB500-170AF594 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 594.



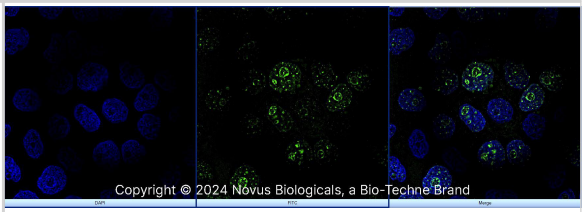
Western Blot: Ki67/MKI67 Antibody [NB500-170] - Ki-67/MKI67 Antibody [NB500-170] - Analysis of A431 (A) and Hek293 (B) cell lysate using Ki67 antibody (NB500-170) at 2 ug/ml.	
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody [NB500-170] - Ki67 antibody was tested at 1:25 in HeLa cells with FITC (green). Nuclei and actin were counterstained with Dapi (blue) and Phalloidin (red).	
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody [NB500-170] - Confocal immunofluorescent analysis of MCF7 cells using Ki67 antibody (NB500-170, 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: Ki67/MKI67 Antibody [NB500-170] - 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- NB500-170 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 [NB500-170] - Ki-67/MKI67 Antibody [NB500-170] - Human tonsil.

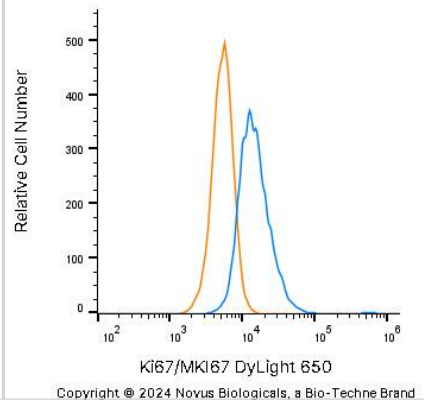


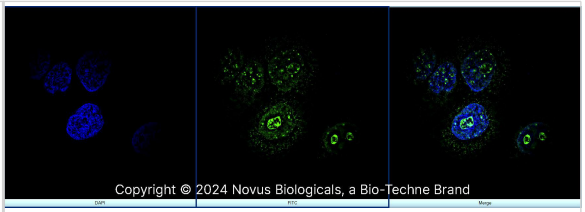
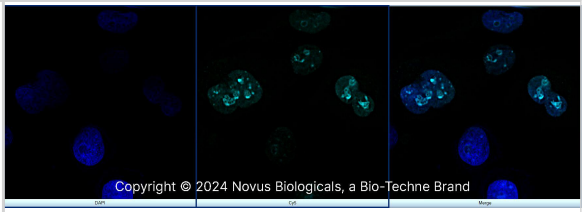
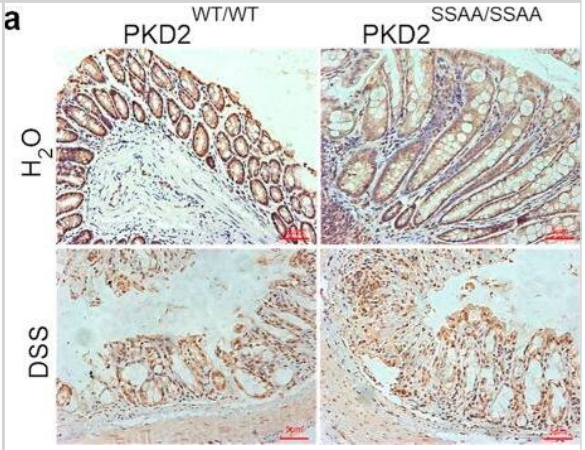
Ki67/MKI67 was detected in immersion fixed MCF7 human breast cancer cell line using Rabbit anti-Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to Biotin (Catalog # NB500-170B) at 5 µg/mL overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

Ki67/MKI67 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti- Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NB500-170AF488) (green) at 2 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

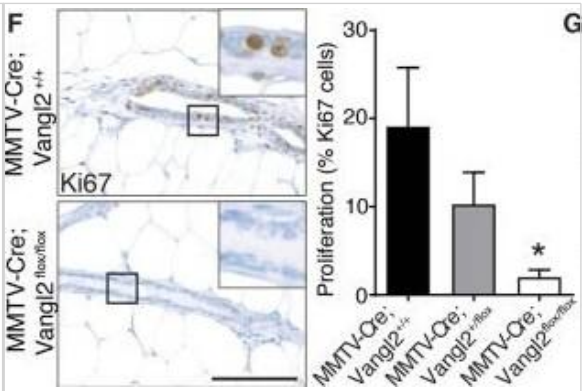


An intracellular stain was performed on A431 human skin carcinoma cell line using Rabbit anti-Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to DyLight 650 (Catalog # NB500-170C, blue histogram) or matched control antibody (orange histogram) at 2.5 µg/mL for 30 minutes at RT.

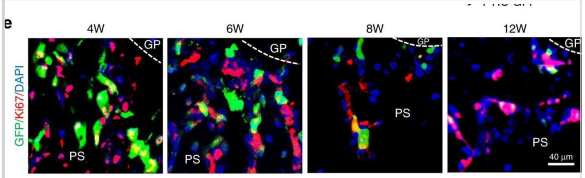


Ki67/MKI67 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti- Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to DyLight 488 (Catalog # NB500-170G) (green) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Ki67/MKI67 was detected in immersion fixed U-251 MG human glioblastoma cell line using Rabbit anti- Ki67/MKI67 Affinity Purified Polyclonal Antibody conjugated to DyLight 650 (Catalog # NB500-170C) (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.	
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB500-170] - PKD2 enzymatic deficiency had no effect on the proliferation or apoptosis of intestinal epithelial cells in mice exposed to DSS.(a) Representative 200× immunohistochemical images (left) & quantification of Ki-67+ proliferating intestinal epithelial cells (right) in wild-type & PKD2SSAA/SSAA mice. Bar represents 5 µm. (b) Representative 200× immunohistochemical images (left) & quantification of cleaved Caspase-3+ apoptotic intestinal epithelial cells (right) in wild-type & PKD2SSAA/SSAA mice. Bar represents 5 µm. Results are representative of three separate experiments with three mice per group. All p > 0.05 as measured by one way-ANOVA among four groups. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep34079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

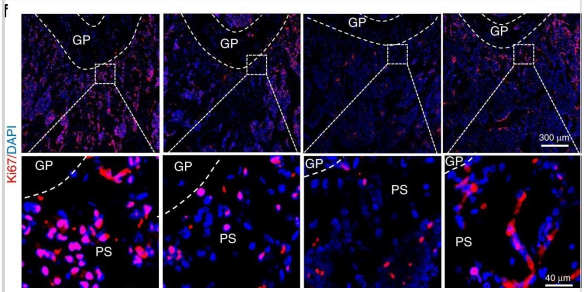
Immunohistochemistry: Ki67/MKI67 Antibody - BSA Free [NB500-170] - Deletion of Vangl2, but not Vangl1, in the mammary gland results in narrow ducts & low cell turnover. (A) RT-qPCR analysis of Vangl1 & Vangl2 mRNA levels in the mammary glands of MMTV-Cre, MMTV-Cre;Vangl2flox/+ & MMTV-Cre;Vangl2flox/flox at 10 weeks of age. (B) Representative images of 10 week old, carmine stained mammary glands & quantification of total branch number from MMTV-Cre, MMTV-Cre;Vangl2flox/+ & MMTV-Cre;Vangl2flox/flox mice (n = 3 mice per genotype). (C) Histological analysis by H&E staining show normal duct formation in 10 week old MMTV-Cre & MMTV-Cre;Vangl2flox/+ glands, whereas ducts from MMTV-Cre;Vangl2flox/flox glands are narrow & have significantly diminished lumens (n = 3 mice/genotype). (D) Immunostaining in mammary ducts show normal distribution of luminal (Cytokeratin 8 (K8), green), & basal (K5), red; SMA magenta) cell populations. (E) Immunofluorescence of adhesion junctional protein E-Cadherin (CDH1, green) & apical membrane marker pERM (red). (F,G) Immunostaining in 10 week old MMTV-Cre;Vangl2+/+ & MMTV-Cre;Vangl2flox/flox glands & quantitation of (F) Ki67 & (G) Cleaved Caspase 3 (n = 3–5 mice per genotype). Lu indicates lumen. * indicates non-magnified duct. Data are represented as mean +/− SEM. Scale bar represents 100 µm (C,F,G) 50 µm (D) & 10 µm (E). Student's t-test *p < 0.05 & ****p < 0.0001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31068622), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB500-170] - Senescent MSPCs are characterized by loss of nestin expression. a, b Representative images of GFP immunofluorescence staining (green) & quantitative analysis of GFP+ cells in femoral primary spongiosa from 4, 6, 8, & 12-week-old male Nestin-GFP mice. Images in upper panels in a are lower power w/ boxes outlining area of higher power in lower panels. Numbers of GFP+ cells per mm2 tissue area in primary spongiosa (N. GFP+ cells/PS.Ar) b. 4W, 6W, 8W, & 12W represent 4-, 6-, 8-, & 12-week-old mice, respectively. DAPI stains nuclei blue. c, d Representative images of flow cytometry analysis (c) & % of CD45−GFP+ cells (d) in femoral metaphysis from 4-, 6-, 8-, & 12-week-old male Nestin-GFP mice. e, f Double-immunofluorescence images of femoral metaphysis sections from 4-, 6-, 8-, & 12-week-old male Nestin-GFP mice using antibodies against GFP (green) & Ki67 (red) e. DAPI stains nuclei blue. GP growth plate. PS primary spongiosa. Quantification of % of GFP+ cells that express Ki67 f. Five mice per group. Data are represented as mean ± s.e.m. *P < 0.05 as determined by ANOVA. g Diagram showing isolation of Nestin-GFP+ (red) & Nestin-GFP− (purple) mesenchymal stem/progenitor cells (MSPCs) by fluorescence-activated cell sorting. Detailed information on isolation of MSPCs from femoral metaphysis from Nestin-GFP mice are described in Supplementary Fig. 3 & Methods section. h–j The sorted cells cultured, & SA-βGal staining (h), BrdU incorporation (i), & p16INK4a immunostaining (j) performed, & representative images shown. k–m Quantification of % of cells that express SA-βGal (k), BrdU (l), & p16INK4a (m). n = 5. Data are represented as mean ± s.e.m. *P < 0.01 as determined by Student's t-tests Image collected & cropped by CiteAb from following publication (https://pubmed.ncbi.nlm.nih.gov/29101351), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Ki67/MKI67 Antibody - BSA Free [NB500-170] - Cellular senescence occurs in primary spongiosa of long bone during late puberty. a–e Representative senescence-associated β-galactosidase (SA-βGal) staining (blue) & quantitative analysis of SA-βGal+ cells in femoral metaphysis (a–c) & diaphysis (d, e) sections from increasing ages of male mice. 4, 6, 8, & 12W represent 4-, 6-, 8-, & 12-week-old mice, respectively. Images in a are lower power with boxes outlining the area of higher power in b. Numbers of SA-βGal+ cells per mm2 tissue area in primary spongiosa (N. SA-βGal+ cells/PS.Ar) (c) & diaphysis (N. SA-βGal+ cells/DP.Ar) (e). Counterstained with eosin (pink). f, g Representative images of immunofluorescence staining (f) & quantitative analysis of ki67+ (g) cells (red) in femoral primary spongiosa from 4, 6, 8, & 12-week-old male mice. DAPI stains nuclei blue. Images in upper panels in f are lower power with boxes outlining the area of higher power in lower panels. Five mice per group. Data are represented as mean ± s.e.m. Ar tissue area, DP diaphysis, GP growth plate, N number, PS primary spongiosa. *P < 0.01 as determined by ANOVA Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29101351), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Galhenage P, Zhou Y, Perry E, Loc B et Al. Replication stress and defective checkpoints make fallopian tube epithelial cells putative drivers of high-grade serous ovarian cancer Cell Rep 2023-09-20 [PMID: 37729060]

Zhang Y, Liu G, Zeng Q, Wu W et Al. CCL19-producing fibroblasts promote tertiary lymphoid structure formation enhancing anti-tumor IgG response in colorectal cancer liver metastasis Cancer Cell 2024-08-13 [PMID: 39137726]

Xi G, Feng P, Zhang X et Al. iPSC-derived cells stimulate ABCG2(+)/NES(+) endogenous trabecular meshwork cell proliferation and tissue regeneration Cell Prolif 2024-02-14 [PMID: 38356373]

Cheng M, Chen S, Li K et Al. CD276-dependent efferocytosis by tumor-associated macrophages promotes immune evasion in bladder cancer Nat Commun 2024-04-01 [PMID: 38561369]

Ofek P, Yeini E, Arad G et al. Deoxyhypusine hydroxylase: A novel therapeutic target differentially expressed in short-term vs long-term survivors of glioblastoma International journal of cancer 2023-05-04 [PMID: 37141410]

Chang TT, Lin LY, Chen C et Al. CCL4 contributes to aging related angiogenic insufficiency through activating oxidative stress and endothelial inflammation Angiogenesis 2024-05-13 [PMID: 38739303]

Tian S, Paudel D, Hao F et al. Refined fiber inulin promotes inflammation-associated colon tumorigenesis by modulating microbial succinate production Cancer reports (Hoboken, N.J.) 2023-07-25 [PMID: 37489647]

Xiong G, Xie N, Nie M et Al. Single-cell transcriptomics reveals cell atlas and identifies cycling tumor cells responsible for recurrence in ameloblastoma Int J Oral Sci 2024-02-29 [PMID: 38424060]

Xiong G, Chen Z, Liu Q et Al. CD276 regulates the immune escape of esophageal squamous cell carcinoma through CXCL1-CXCR2 induced NETs J Immunother Cancer 2024-01-01 [PMID: 38724465]

Wang X, Ling R, Peng Y et Al. RNPS1 stabilizes NAT10 protein to facilitate translation in cancer via tRNA ac(4)C modification Int J Oral Sci 2024-01-22 [PMID: 38246918]

Dsilva A, Avlas S, Rhone N et Al. A Mouse Model for Eosinophilic Esophagitis (EoE) Curr Protoc 2024-02-20 [PMID: 38372429]

Zhu M, Fang Y, Cheng Y et Al. The Alleviating Effect of Taxifolin on Deoxynivalenol-Induced Damage in Porcine Intestinal Epithelial Cells Vet Sci 2024-03-30 [PMID: 38668423]

More publications at http://www.novusbio.com/NB500-170

Procedures

Immunocytochemistry/Immunofluorescence Protocol for Ki67/MKI67 Antibody (NB500-170)
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 Ki67/MKI67 Antibody (NB500-170)
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.

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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