Product Datasheet

GFP Antibody - BSA Free
NB100-1614

Unit Size: 0.1 ml

Store at -20C in the dark. Avoid freeze-thaw cycles.

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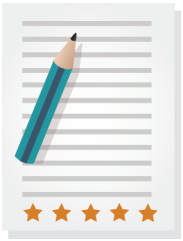
Updated 2/21/2025 v.20.1



NB100-1614

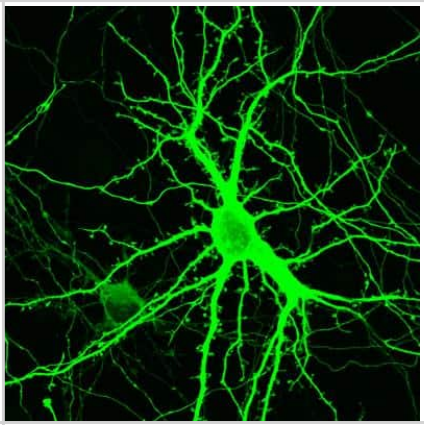
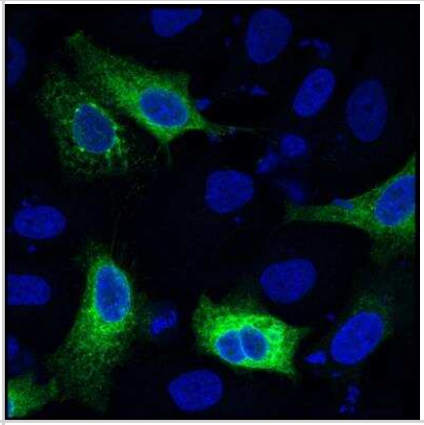
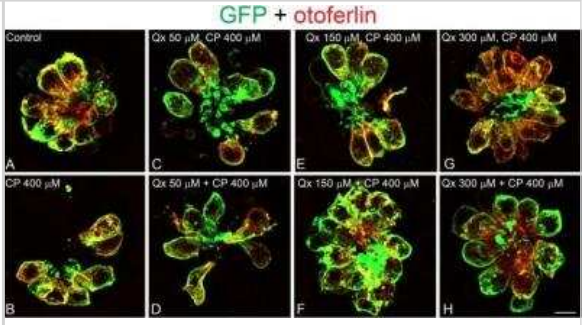
GFP Antibody - BSA Free

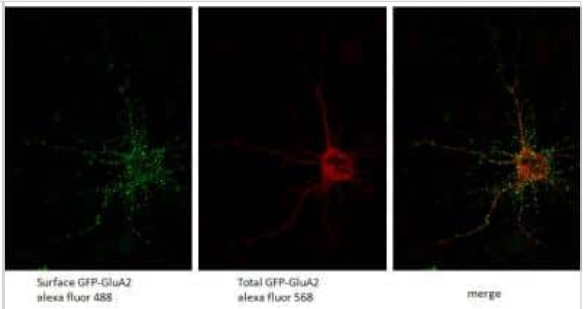
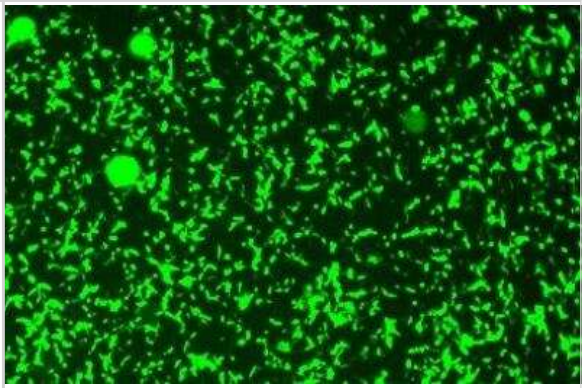
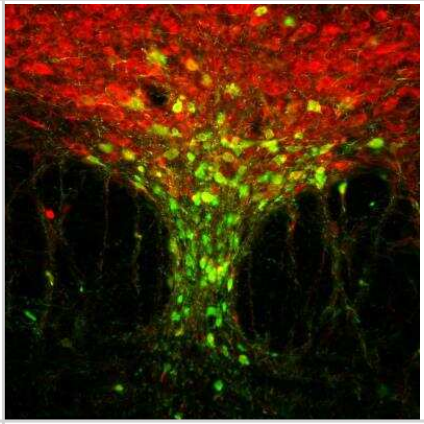
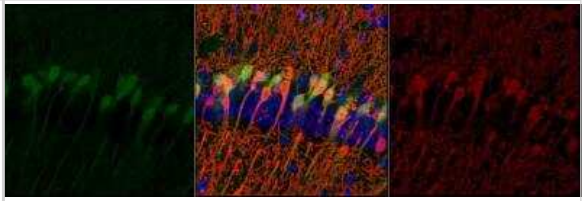
Product Information	
Unit Size	0.1 ml
Concentration	10.0 mg/ml
Storage	Store at -20C in the dark. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgY
Purity	Immunogen affinity purified
Buffer	10 mM PBS (0.9% isotonic, w/v, pH 7.2) and 50% (v/v) Glycerol

Product Description	
Host	Chicken
Species	Non-species specific
Reactivity Notes	NB100-1614 has been tested on transgenic mice expressing recombinant GFP.
Immunogen	This GFP antibody was developed by immunizing chickens with purified recombinant green fluorescent protein (GFP) emulsified in Freund's adjuvant.
Notes	Chicken products cannot be exported to Canada. Purification Notes Chickens were immunized with purified recombinant green fluorescent protein (GFP) emulsified in Freund's adjuvant. After multiple injections, eggs were collected from the hens, and IgY fractions were prepared from the yolks and then affinity-purified antibodies were prepared using GFP conjugated to an agarose matrix. The final product is a filter-sterilized mixture of both affinity-purified antibodies (30 ug/ml) and purified IgY (10 mg/mL). Storage Notes Store at -20C in the dark. Under these conditions, the antibodies should have a shelf life of at least 12 months (provided they remain sterile). Since 50% glycerol is present in the vial, this antibody preparation should remain a liquid at -20C. For longer storage periods, store at -80C, but be aware that freezing this preparation may reduce its activity.

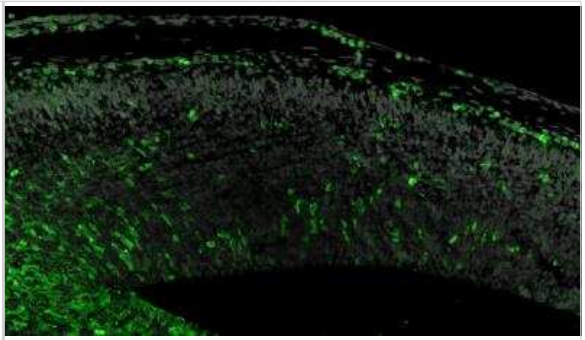
Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, Immunohistochemistry Free-Floating
Recommended Dilutions	Western Blot 1:5000 - 1:10000, Flow Cytometry, ELISA 1:100 - 1:2000, Immunohistochemistry 1:2000 - 1:5000, Immunocytochemistry/ Immunofluorescence 1:2000-1:5000, Immunohistochemistry-Paraffin 1:10 - 1:500, Immunohistochemistry-Frozen 1:10 - 1:500, In vivo assay, Immunohistochemistry Free-Floating
Application Notes	Use in In vivo reported in scientific literature (PMID:34911937).Use in IHC-FrFl reported in scientific literature (PMID:33558651). Use in IHC-F was reported in the scientific literature (PMID: 23799397).

Images

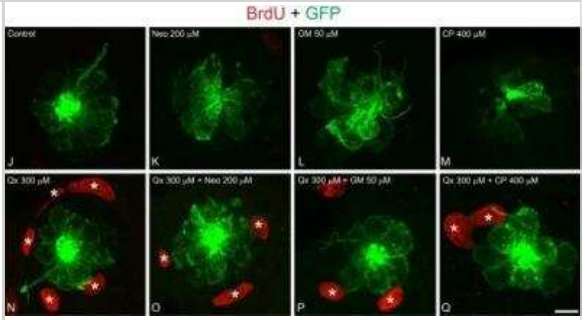
Immunocytochemistry/Immunofluorescence: GFP Antibody [NB100-1614] - Staining of cultured hippocampal neuron from rat. ICC/IF image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: GFP Antibody [NB100-1614] - HeLa cells transiently expressing GFP were visualized using GFP antibody (green). Nuclei were counterstained with DAPI. ICC/IF image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: GFP Antibody [NB100-1614] - IF analysis of GFP in mouse trigeminal ganglia, cornea. Image courtesy of product review by Jessica Newsom.	
Immunohistochemistry: GFP Antibody [NB100-1614] - 5dpf Tg (brn3c:GFP) larvae were incubated with vehicle alone (DMSO, A), 400uM of cisplatin (CP, B), pre-treated with Qx for 2 hours and then incubated with CP for 6 hours (Qx, CP, C,E,G) or pre-treated with Qx for 2 hours and then co-treated with Qx and CP for 6 more hours (Qx+CP, D,F,H). Animals were fixed and immunostained for GFP (green) and otoferlin (red). Image collected and cropped by CiteAb from the following publication (nature.com/articles/s41598-018-33520-w), licensed under a CC-BY license.	

Immunocytochemistry/Immunofluorescence: GFP Antibody [NB100-1614] - IF analysis of GFP in rat cortical culture (2 weeks old). ICC/IF image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: GFP Antibody [NB100-1614] - LNCaP human prostate cancer cell line imaged after transfection with GFP expressing plasmid. ICC/IF image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: GFP Antibody [NB100-1614] - Staining of a tissue section through the midbrain region of an adult mouse for GFP (green) and Tryptophan Hydroxylase-positive neurons (RED).	
Immunohistochemistry-Paraffin: GFP Antibody [NB100-1614] - Pyramidal neurons in the hippocampal formation of a neonatal mouse brain. Tissue was paraformaldehyde-fixed (4%) and paraffin-embedded. GFP staining is in green in left panel.	

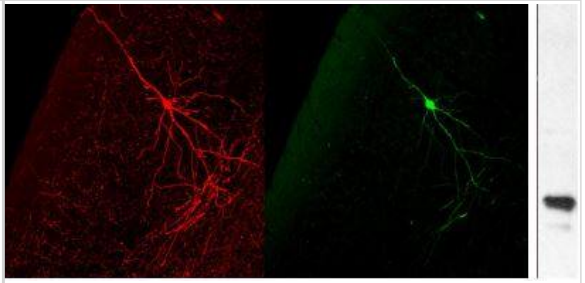
Immunohistochemistry-Paraffin: GFP Antibody [NB100-1614] - Embyonic mouse brain section. GFP in green, DAPI in grey. IHC-P image submitted by a verified customer review.



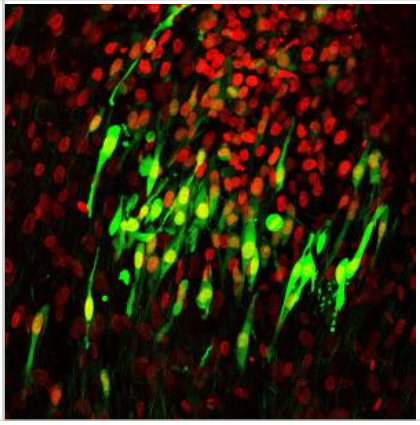
Immunohistochemistry: GFP Antibody [NB100-1614] - (J) control. (K) Neo 200uM 30min. (L) GM 50uM 1 hour. (M) CP: 400uM 2 hours. (N) Qx 300uM 8 hours. (O) Qx 300uM 8 hours + Neo 200uM 30min. (P) Qx 300uM 8 hours + GM 50uM 1 hour. (Q) Qx 300uM 8 hours + CP 400uM 2 hours. Asterisks denote neuromast supporting cells positive for BrdU. Image collected and cropped by CiteAb from the following publication (nature.com/articles/s41598-018-33520-w), licensed under a CC-BY license.



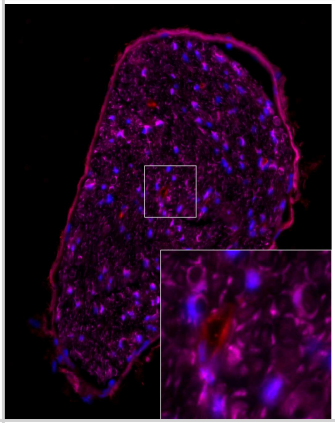
Comparison between GFP-immunoreactivity using NB100-1614 (left panel in red) and autofluorescence (right panel in green). In this case, the cortical neuron in this unfixed thick section was first photographed for GFP autofluorescence (left), and then the section was fixed (4% paraformaldehyde) and immunostained for GFP-immunoreactivity (1:1000 dilution) using Texas Red-goat anti-chicken IgY antibodies as a secondary. The same cell (left) was then identified. Far right: Western blot showing specific immunolabeling of the GFP protein.



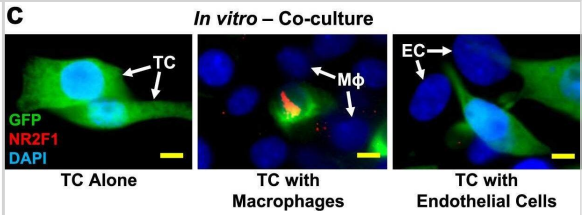
Immunohistochemistry: Chicken Polyclonal GFP Antibody [NB100-1614] - Chicken Polyclonal GFP Antibody on mouse cancer tissue. DAPI (Red) and Cytopastmic GFP stain (Green). Primary antibody dilution: 1:200 in a 200 um slice. Image from a verified customer review.



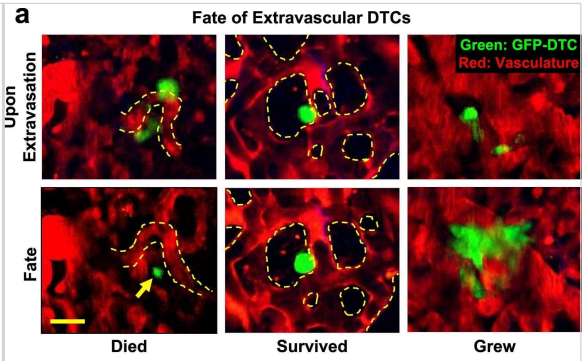
Immunohistochemistry-Frozen: Chicken Polyclonal GFP Antibody [NB100-1614] - Image depicting GFP in magenta and CD31 (Catalog # AF3628) in red. Tissue is a fixed frozen section of sciatic nerve obtained from a S100A4 GFP mouse. NB100-1614 was diluted 1 in 10000 and was left on tissue sections overnight at 4 degrees Celsius. Secondary antibody was donkey anti-chicken conjugated to Alexa 647. Image from a verified customer review.



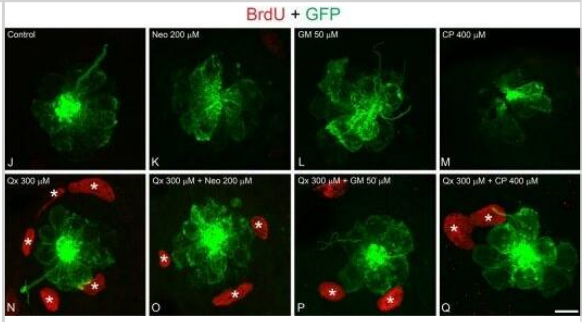
Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Macrophages regulate dormancy in tumor cells.a Representative image of triple immunofluorescently stained in E0771-GFP primary tumor tissue for tumor cells, macrophages, & NR2F1. Green = GFP; Red = NR2F1; White = IBA-1; Blue = DAPI. White arrow shows a macrophage. The yellow arrow contact between an NR2F1-positive tumor cell & a macrophage. Mϕ=Macrophage. Scale bar=20 µm. b Quantification showing frequency of distances between NR2F1+ tumor cells to nearest macrophage in primary tumor. Data is normalized to frequency of distances between all DAPI+ nuclei to nearest TMEM. Bar = mean. Error bars = ±SEM. n = 34 fields of view (551 × 316 µm2) in 4 animals. For comparison between 0 & 200 µm bins a two-tailed Mann-Whitney test used ($p < 0.0001$). ****$p < 0.0001$. c Representative immunofluorescence images of NR2F1 expression in E0771-GFP tumor cells cultured alone, in direct contact w/ BAC1.2F5 macrophages,/in direct contact w/ HUVEC endothelial cells. White arrows show macrophages/endothelial cells in direct contact w/ a tumor cell. Green = GFP; Red = NR2F1; Blue = DAPI. TC = Tumor Cell. Mϕ = Macrophage. EC = Endothelial Cell. Scale bar = 15 µm. d Percentage of NR2F1-positive tumor cells from each group in C. TC alone: n = 777 cells in 9 independent experiments; TC+Mϕ; n = 226 cells in 6 independent experiments, TC+EC = n = 359 cells in 4 independent experiments. Bar = mean. Error bars = ±SEM. For TC vs. TC+Mϕ ($p = 0.0039$), & for TC vs. TC+EC ($p = 1$), a two-tailed Kruskal-Wallis test w/ Dunn's multiple comparisons adjustment used. For TC+Mϕ vs. TC+EC (0.012), a two-tailed one-way ANOVA w/ Sidak's multiple comparison adjustment used. *$p < 0.05$. **$p < 0.01$; ns = not significant. Source data are provided as a Source Data file. Image collected & cropped by CiteAb from following publication (https://pubmed.ncbi.nlm.nih.gov/35110548), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



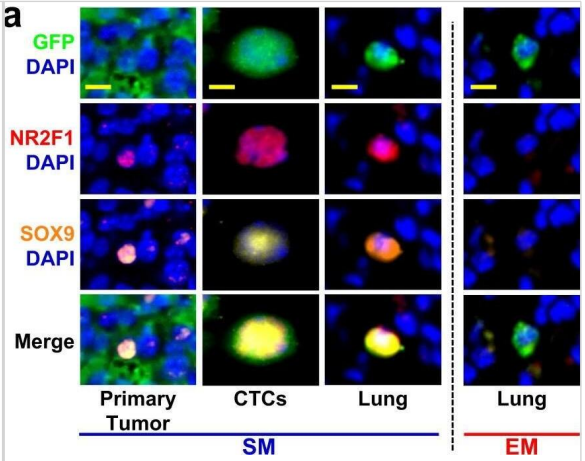
Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Spontaneously metastasizing tumor cells survive significantly longer at the secondary site compared to intravenously injected tumor cells.a Representative intravital microscopy images showing the possible fates of extravascular disseminated tumor cells in the lung parenchyma. Top: Images of disseminated tumor cells just after extravasation. Bottom left: Example of an extravascular tumor cell, which has died, as evidenced by small extravascular apoptotic bodies (yellow arrow). Bottom middle: Example of an extravascular tumor cell that survived as a single & solitary tumor cell over time. Bottom right: Example of an extravascular tumor cell that began to divide & grow into a micro-metastasis. Red = tdTomato labeled endothelial cells & 155 kDa Tetramethylrhodamine dextran labeled blood serum, Green = GFP labeled tumor cells. Yellow dashed lines delineate blood vessel boundaries. Scale bar = 15 µm. b Percentage of extravascular E0771-GFP disseminated tumor cells that died, survived, or grew after extravasation in EM & SM models 64 hrs after arrival to the lung vasculature. EM: n = 27 tumor cells in 4 mice. SM: n = 31 tumor cells in 4 mice. Bar = mean. Error bars = ±SEM. For Died & Survived columns, a two-tailed unpaired t-test was used (p = 0.0003 & 0.0005, respectively). For Grew columns, a two-tailed Mann-Whitney test was used (p = 0.14). ***p < 0.001. ns = not significant. Source data are provided as a Source Data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35110548), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



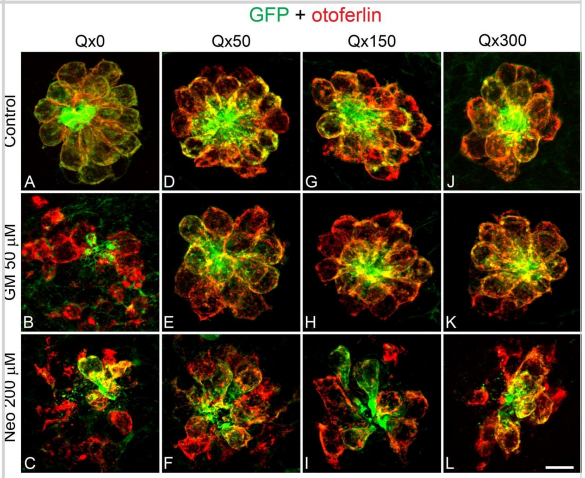
Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Qx protects against ototoxin-induced HC death & promotes supporting cell proliferation. (A–I) TUNEL assay (red) performed in zebrafish incubated w/ vehicle or 300 µM of Qx alone (controls) or w/ corresponding ototoxin w/ or w/out Qx 300 µM. Animals counterstained w/ phalloidin (green). (A) Neomycin (Neo) 200 µM incubation for 30 min. (B) Gentamicin (GM) 50 µM incubation for 1 hour. (C) Cisplatin (CP) 400 µM incubation for 2 hrs. (D) GM 50 µM incubation for 1 hour followed by recovery for 5 hrs. (E) Incubation w/ Qx 300 µM for 8 hrs & Neo 200 µM for 30 min. (F) Incubation w/ Qx 300 µM for 8 hrs & GM 50 µM for 1 hour. (G) Qx 300 µM for 8 hrs + CP 400 µM for 2 hrs. (H) Qx 300 µM incubation for 8 hrs + GM 50 µM for 1 hour followed by 5 hrs recovery. Asterisks denote TUNEL-positive HCs. (I) The % of TUNEL-positive neuromasts calculated for each treatment & represented as mean +/− SEM. (J–R) Proliferation assays performed in 5dpf Tg(brn3c:GFP) in presence/absence of Qx & corresponding ototoxin, by BrdU-labelling method (red). Animals immunostained for GFP (green). (J) control. (K) Neo 200 µM 30 min. (L) GM 50 µM 1 hour. (M) CP: 400 µM 2 hrs. (N) Qx 300 µM 8 hrs. (O) Qx 300 µM 8 hrs + Neo 200 µM 30 min. (P) Qx 300 µM 8 hrs + GM 50 µM 1 hour. (Q) Qx 300 µM 8 hrs + CP 400 µM 2 hrs. Asterisks denote neuromast supporting cells positive for BrdU. (R) The % of BrdU-positive supporting cells per neuromast calculated for each treatment & represented as mean +/− SEM. One-way ANOVA, *p < 0.05, **p < 0.01. Black asterisks compared versus corresponding control. Red asterisk compared versus corresponding ototoxin-only treatment. Scale bar: (A–H) 10 µm, (J–O) 7 µm. Data taken from at least 15 animals & 3 experiments runs. Image collected & cropped by CiteAb from following publication (https://pubmed.ncbi.nlm.nih.gov/30310154), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



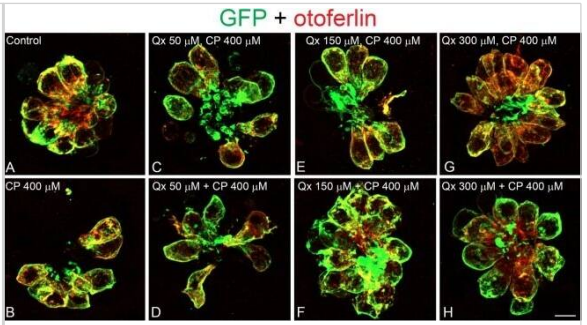
Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Spontaneously metastasizing tumor cells are more frequently doubly positive for dormancy & stem-like markers compared to intravenously injected tumor cells.a Representative images of triple immunofluorescence staining for GFP, NR2F1, & SOX9 expression in primary tumors, circulating tumor cells (CTCs), & disseminated tumor cells (Lung) from an E0771-GFP SM model (Left) & in disseminated tumor cells (Lung) from an EM model (Right). Green = GFP; Red = NR2F1; Orange = SOX9; Blue = DAPI. Scale bar for Primary Tumor = 50 µm. Scale bar for CTCs & Lung = 15 µm. b Percentage of double-positive tumor cells NR2F1-positive SOX9High from each group in Fig. 5a. Primary Tumor: n = 2383 in 97 fields of view (65 × 65 µm2) in 7 animals; CTCs: n = 379 cells in 8 animals; SM Lung: n = 104 cells in 9 animals; In vitro: n = 413 cells in 3 independent experiments. EM Lung: n = 75 cells in 7 animals. Bar = mean. Error bars = ±SEM. For EM Lung vs. SM Lung ($p = 0.0001$) & EM Lung vs. in vitro ($p = 0.69$), a two-tailed Kruskal-Wallis test with Dunn's multiple comparisons adjustment was used. For PT vs. CTC: ($p = 0.0041$), PT vs. Lung SM ($p = 0.0030$), & CTC vs. Lung SM ($p = 1.00$) a two-tailed ANOVA test with Sidak's multiple comparisons adjustment was used. **p < 0.01. ***p < 0.001. ns = not significant. Source data are provided as a Source Data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35110548), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



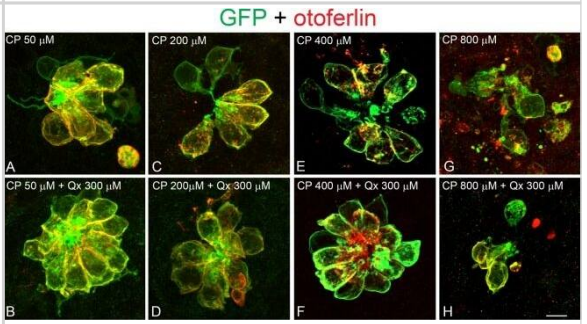
Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Dose protection curve against aminoglycosides. 5dpf larvae were incubated with vehicle (E3, A) or with 50 µM (D–F), 150 µM (G–I) or 300 µM (J–L) of Qx for a total of 8 hours. Gentamicin (GM, 50 µM, B,E,H,K) or neomycin (Neo, 200 µM,C,F,I,L) were added during the last 60 min or 30 min of incubation, respectively. Animals were fixed & stained for otoferlin (red) & GFP (green). (M) Quantification of the number of hair cells per neuromast after the different treatments represented as mean +/− SEM. Note that since no significant differences were found in the number of hair cells per neuromast when animals were incubated with the different Qx concentrations (0–300 µM, A,D,G,J), the control value represents the average of all these treatments. One-way ANOVA, Dunnett post test. *p < 0.05, **p < 0.01, ***p < 0.001. Black asterisks compared versus control. Red asterisks compared versus the corresponding aminoglycoside-only treatment. (N) Scores for neuromast morphology (see Materials & Methods). Scale bar: 7 µm. Data were taken from at least 20 animals & 3 experiments runs. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30310154), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Qx protects against cisplatin ototoxicity. 5dpf Tg(brn3c:GFP) larvae were incubated with vehicle alone (DMSO, A), 400 µM of cisplatin (CP, B), pre-treated with Qx for 2 hours & then incubated with CP for 6 hours (Qx, CP, C,E,G) or pre-treated with Qx for 2 hours & then co-treated with Qx & CP for 6 more hours (Qx + CP, D,F,H). Animals were fixed & immunostained for GFP (green) & otoferlin (red). (I) Quantification of the number of hair cells per neuromast after the different treatments represented as mean +/− SEM. One-way ANOVA, Dunnett post test. *p < 0.05, ***p < 0.001. Black asterisks compared versus control. Red asterisks compared versus CP 400 µM. (J) Scores for neuromast morphology (see Materials & Methods). Scale bar: 6 µm. Data were taken from at least 20 animals & 3 experiments runs. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30310154), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: GFP Antibody [NB100-1614] - Dose protection curve against CP. 5dpf Tg(brn3c:GFP) larvae were incubated with 50 µM to 800 µM of CP (A,C,E,G) for 6 hours or pre-treated with 300 µM of Qx for 2 hours & then co-treated with Qx & CP (50µM-800µM) for 6 hours (B,D,F,H). Animals were fixed & immunostained for GFP (green) & otoferlin (red). Control animals were exposed to vehicle alone (DMSO). (I) Quantification of the number of hair cells per neuromast after the different treatments represented as mean +/− SEM. One-way ANOVA, Dunnett post test. ***p < 0.001. Black asterisks compared versus DMSO-treated animals. Red asterisks compared versus the corresponding CP concentration. (J) Scores for neuromast morphology (see Materials & Methods). Scale bar: 6 µm. Data were taken from at least 20 animals & 3 experiments runs. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30310154), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

D'Souza SP, Upton BA, Eldred KC, Glass I et Al. Developmental control of rod number via a light-dependent retrograde pathway from intrinsically photosensitive retinal ganglion cells Dev Cell 2024-08-14 [PMID: 39142280]

Vijayakumar S, DiGuiseppi JA, Dabestani PJ, Ryan WG et Al. In silico transcriptome screens identify epidermal growth factor receptor inhibitors as therapeutics for noise-induced hearing loss Sci Adv 2024-06-19 [PMID: 38896614]

Yang D, Jacobson A, Meerschaert KA, Sifakis JJ et Al. Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection Cell 2022-10-15 [PMID: 36243004]

Perniss A, Liu S, Boonen B et Al. Chemosensory Cell-Derived Acetylcholine Drives Tracheal Mucociliary Clearance in Response to Virulence-Associated Formyl Peptides Immunity 2020-11-04 [PMID: 32294408]

Keshavarz M, Ruppert AL, Meiners M et Al. Bitter tastants relax the mouse gallbladder smooth muscle independent of signaling through tuft cells and bitter taste receptors Sci Rep 2024-08-08 [PMID: 39117690]

Kawatani M, de Groat WC, Itoi K et Al. Downstream projection of Barrington's nucleus to the spinal cord in mice J Neurophysiol 2022-03-10 [PMID: 34731061]

Parham LR, Williams PA, Katada K et Al. IGF2BP1/IMP1 Deletion Enhances a Facultative Stem Cell State via Regulation of MAP1LC3B Cell Mol Gastroenterol Hepatol 2024-01-01 [PMID: 38081361]

Yan, Y;Li, X;Gao, Y;Mathivanan, S;Kong, L;Tao, Y;Dong, Y;Li, X;Bhattacharyya, A;Zhao, X;Zhang, SC; 3D bioprinting of human neural tissues with functional connectivity Cell Stem Cell 2024-02-01 [PMID: 38306994]

Chinn HK, Gardell JL, Matsumoto LR et al. Hypoxia-inducible lentiviral gene expression in engineered human macrophages Journal for immunotherapy of cancer 2022-06-01 [PMID: 35728871]

S Swarnkar, Y Avchalumov, I Espadas, E Grinman, XA Liu, BL Raveendra, A Zucca, S Mediouni, A Sadhu, S Valente, D Page, K Miller, SV Puthanveet Molecular motor protein KIF5C mediates structural plasticity and long-term memory by constraining local translation Cell Reports, 2021-07-13;36(2):109369. 2021-07-13 [PMID: 34260917] (Chromatin Immunoprecipitation)

Ambroziak, W;Nencini, S;Pohle, J;Zuza, K;Pino, G;Lundh, S;Araujo-Sousa, C;Goetz, LIL;Schrenk-Siemens, K;Manoj, G;Herrera, MA;Acuna, C;Siemens, J; Thermally induced neuronal plasticity in the hypothalamus mediates heat tolerance Nature neuroscience 2024-12-09 [PMID: 39653806]

Lee M, Carpenter C, Hwang YS et al. Proliferation associated 2G4 is required for the ciliation of vertebrate motile cilia Communications Biology 2024-11-04 [PMID: 39496919]

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Procedures

Immunohistochemistry Chicken IgY Protocol (NB100-1614)

Citrate Buffer Antigen Retrieval Protocol

Background: Formaldehyde fixation (2% or 4%, or as a component of 10% formalin) produces protein cross-links in tissues that tends to interfere with antibody penetration. This seems to be particularly true of paraffin- embedded formaldehyde-fixed tissue. Since chicken IgY antibodies are larger than rabbit or mouse IgG's, "extra steps" may be necessary to compensate for their larger size.

The citrate-based "antigen retrieval" protocol outlined below has been shown to improve chicken IgY antibody penetration into 4% formalde- hyde-fixed paraffin-embedded sections, and can increase the degree and intensity of immunoreactivity and immunostaining.

Reagents (NOTE: You can use either the Sodium Citrate or Citric Acid Buffers in step #3, below)

"Sodium Citrate Buffer" (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)

Weigh out 2.94 grams of trisodium citrate (dihydrate). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.00 with 1.0 N HCl. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Citric Acid Buffer" (10mM Citric Acid, 0.05% Tween 20, pH 6.0)

Weigh out 1.92 grams of citric acid (anhydrous). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.0 with 1.0 N NaOH. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Phosphate-Buffered Saline" [PBS, 10 mM Sodium phosphate-buffered (pH 7.2) isotonic (0.9%, w/v) saline solution] PBS Tween (0.05% Tween 20 in PBS)
Ethanol (80%, 90%, 95%, 100%) diluted with water

Xylene

Procedure (for use with paraffin-embedded sections):

1 Deparaffinize tissue sections in 2 changes of xylene (5 minutes each).

2. Hydrate in 2 changes of 100% ethanol (3 minutes each), 95% ethanol (1 minute), 90% ethanol (1 minute), 80% ethanol (1 minute). Rinse in distilled water.

3. Pre-heat steamer or water bath with staining dish containing either Sodium Citrate Buffer or Citrate Buffer. Wait until temperature reaches 95-100 degrees C.

NOTE: Microwave or pressure cooker can be used as an alternative as a heating source.

4. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes (optimal incubation times will vary).

5. Remove the staining dish, and allow it to cool to room temperature (for 20 minutes or so).

6. Rinse sections in PBS Tween twice for 2 minutes each time.

NOTE: The remainder of this protocol is meant to be a suggestion, and can be substituted with your regular immunostaining protocol.

7. Block sections for 30 minutes with Blocking buffer diluted 1:10 with water.

8. Incubate sections with primary antibody at appropriate dilution in antibody dilution buffer overnight at 4 degrees C. Since chicken IgY antibodies are larger than mammalian IgG's, this overnight incubation allows more time for antibody penetration into tissue sections.

9. Rinse sections with PBS Tween 20 twice for 5 minutes each time.

10. Incubate sections with labeled secondary antibody (see NOTE, below) at appropriate dilution (for one hour at room temperature) in a 1:100 dilution of blocking buffer (diluted in PBS).

11. Rinse with PBS Tween 20 for three times for 5 minutes each time.

NOTE: This protocol may use HRP- or fluorescently-labeled secondary antibodies produced in goats or rabbits.

References:

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