abcam

Product datasheet

Anti-beta III Tubulin antibody [2G10] - Neuronal Marker ab78078

KO VALIDATED

★★★★★ 40 Abreviews 259 References 16 Images

Overview

Product name	Anti-beta III Tubulin antibody [2G10] - Neuronal Marker
Description	Mouse monoclonal [2G10] to beta III Tubulin - Neuronal Marker
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IHC-P, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human, Common marmoset, Dogfish, Catshark **Predicted to work with:** Rabbit, Chicken, Cow, Cat, Quail
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1 , HeLa, HEK-293 cell lysates; Human, mouse and rat brain tissue lysate. IHC-P: Human, marmoset and rat cerebellum, mouse brain, human medulla oblongata. ICC/IF: NGF-differentiated PC12 cells, mouse differentiated neural stem cells, Primary rat neurons/glia, DIV14 cells. Flow Cyt-Intra: SH-SY5Y cells.
General notes	This antibody clone [2G10] is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact **orders@abcam.com** or you can find further information **here**. Abcam recommended secondaries - Goat Anti-Mouse HRP (**ab205719**) and Goat Anti-Mouse Alexa Fluor® 488 (**ab150113**). See other **anti-mouse secondary antibodies** that can be used with this antibody. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid

Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	2G10
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab78078 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

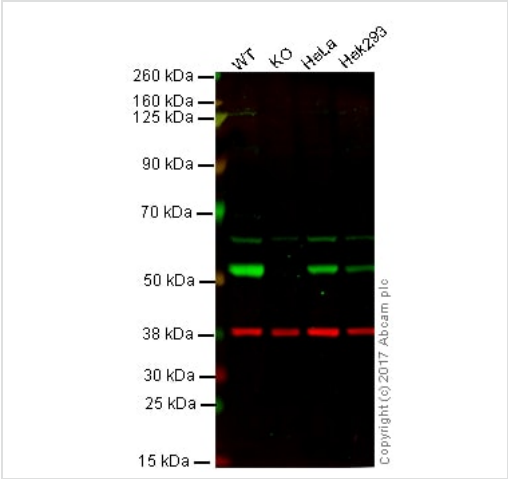
Application	Abreviews	Notes
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (8)	Use a concentration of 1 µg/ml.
IHC-P	★★★★★ (17)	Use a concentration of 0.2 - 1 µg/ml.
IP		Use at an assay dependent concentration.
WB	★★★★★ (3)	Use a concentration of 1 µg/ml. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).

Target

Function	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. TUBB3 plays a critical role in proper axon guidance and mantainance.
Tissue specificity	Expression is primarily restricted to central and peripheral nervous system.
Involvement in disease	Defects in TUBB3 are the cause of congenital fibrosis of extraocular muscles type 3A (CFEOM3A) [MIM:600638]. A congenital ocular motility disorder marked by restrictive ophthalmoplegia affecting extraocular muscles innervated by the oculomotor and/or trochlear nerves. It is clinically characterized by anchoring of the eyes in downward gaze, ptosis, and backward tilt of the head. Congenital fibrosis of extraocular muscles type 3 presents as a non-progressive, autosomal dominant disorder with variable expression. Patients may be bilaterally or unilaterally affected, and their oculo-motility defects range from complete ophthalmoplegia (with the eyes fixed in a hypo- and exotropic position), to mild asymptomatic restrictions of ocular movement. Ptosis, refractive error, amblyopia, and compensatory head positions are associated with the more severe forms of the disorder. In some cases the ocular phenotype is accompanied by additional features including developmental delay, corpus callosum agenesis, basal ganglia

	dysmorphism, facial weakness, polyneuropathy.
Sequence similarities	Belongs to the tubulin family.
Domain	The highly acidic C-terminal region may bind cations such as calcium.
Post-translational modifications	Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs exclusively on glutamate residues and results in polyglutamate chains on the gamma-carboxyl group. Also monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella) whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of such modifications is still unclear but they regulate the assembly and dynamics of axonemal microtubules.
Cellular localization	Cytoplasm > cytoskeleton.

Images



Western blot - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

All lanes :

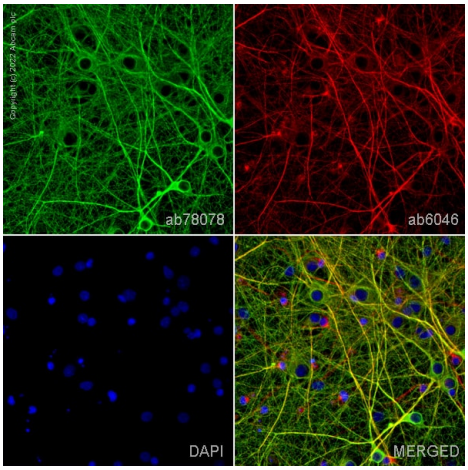
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Beta III Tubulin knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : HEK-293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 50 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab78078 observed at 50 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab78078 was shown to specifically react with beta III Tubulin in wild-type HAP1 cells as signal was lost in beta III Tubulin knockout cells. Wild-type and beta III Tubulin knockout samples were subjected to SDS-PAGE. ab78078 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 10 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

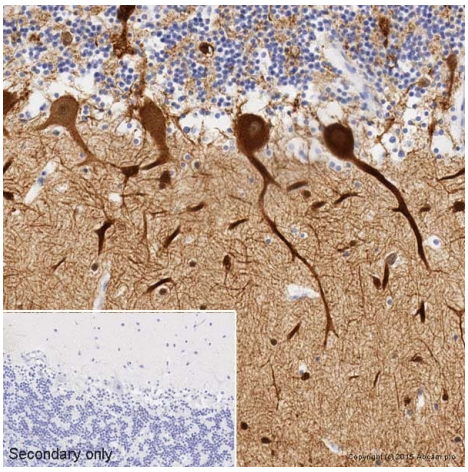


Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

ab78078 staining beta III Tubulin in primary rat neurons/glia, DIV14 (prepared from E18 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDHEP) cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab78078 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

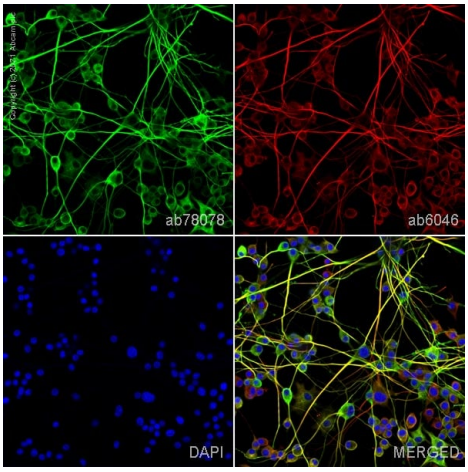
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

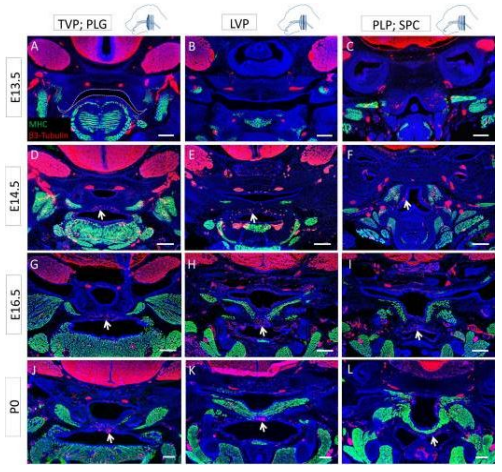
IHC image of ab78078 staining beta III Tubulin in Human cerebellum formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0 epitope retrieval solution 1) for 20 mins. The section was then incubated with ab78078, 0.5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

ab78078 staining beta III Tubulin in NGF-differentiated PC12 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab78078 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



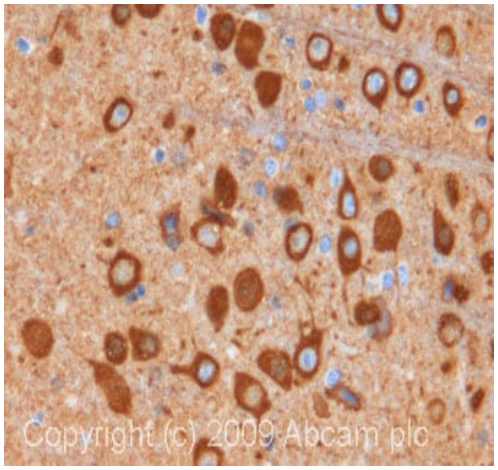
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

Soft palate innervation is primarily oro-medial and complements the differentiated muscle pattern

(A-L) MHC (green) and β3-tubulin (red) co-immunostaining along the antero-posterior axis of the mouse soft palate at E13.5 (A-C), E14.5 (D-F), E16.5 (G-I), and P0 (J-L) at the level of the TVP and PLG (A, D, G, J), LVP (B, E, H, K), and PLP and SPC (C, F, I, L). Dashed lines indicate the outline of the palatal shelf and tongue epithelium. Arrows indicate the nerve fibers innervating the palatal shelves in a pattern complementary to the differentiated muscle, mainly located orally in the medial region. The schematic drawings indicate the orientation and the position of each section. Scale bars: 200µm.

beta III Tubulin is detected with ab78078.

(From Figure 4 of Grimaldi et al)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

IHC image of ab78078 staining in mouse brain formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab78078, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

Western blot - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

All lanes : Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078) at 1 µg/ml

Lane 1 : Human brain tissue lysate - total protein (**ab29466**)
Lane 2 : Brain (Mouse) Tissue Lysate
Lane 3 : Brain (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/50000 dilution

Developed using the ECL technique.

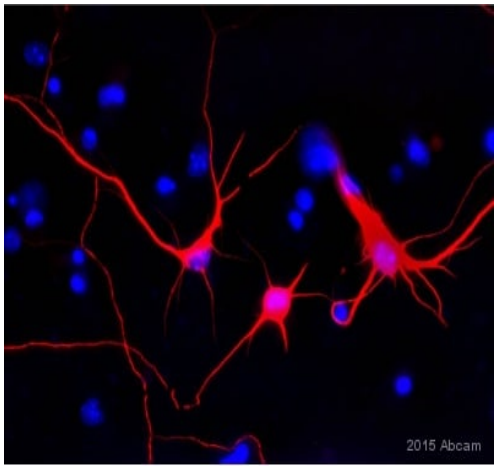
Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 50 kDa

Exposure time: 90 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being

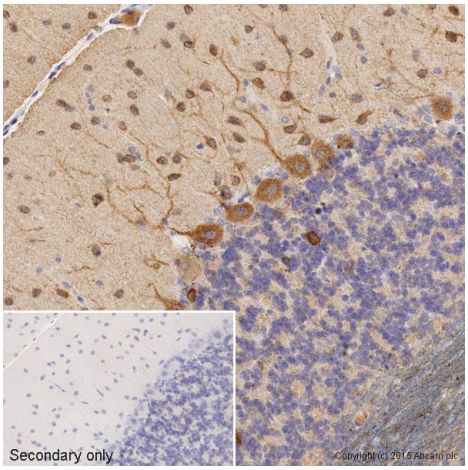
transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab78078 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution **ab133406**



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

This image is courtesy of an anonymous Abreview

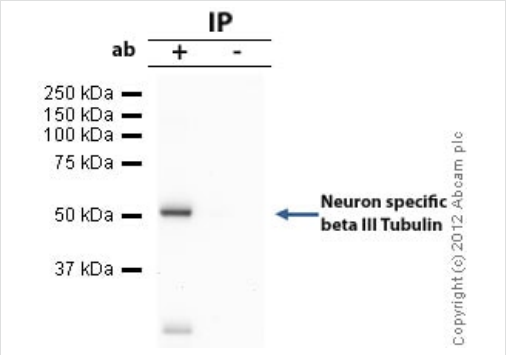
ab78078 staining beta III Tubulin (red) in mouse differentiated neural stem cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.2% Triton X-100. Samples were incubated with primary antibody (5µg/ml in PBS + 3% BSA) for 16 hours at 4°C. An Alexa Fluor® 568-conjugated donkey anti-mouse IgG polyclonal (1/1000) was used as the secondary antibody. Blue - DAPI nuclear counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

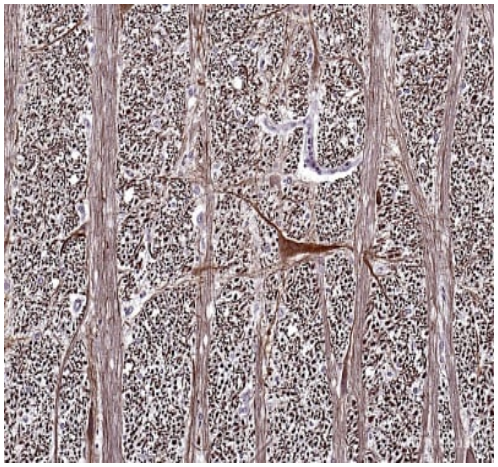
IHC image of ab78078 staining beta III Tubulin in rat cerebellum formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with EDTA/Tris (epitope retrieval solution 2) for 20 mins. The section was then incubated with ab78078, 0.5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunoprecipitation - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

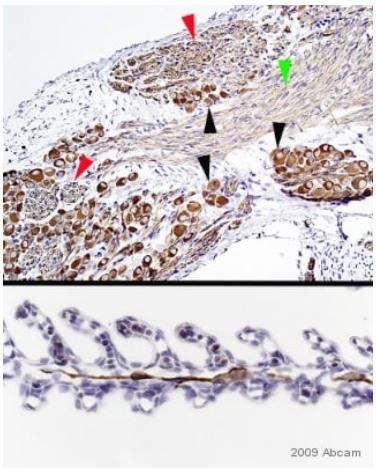
Neuron specific beta III Tubulin - Neuronal Marker was immunoprecipitated using 0.5mg Mouse Brain whole tissue lysate, 5µg of Mouse monoclonal [2G10] to Neuron specific beta III Tubulin - Neuronal Marker and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Mouse Brain whole tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab78078.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: 50kDa: Neuron specific beta III Tubulin - Neuronal Marker.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

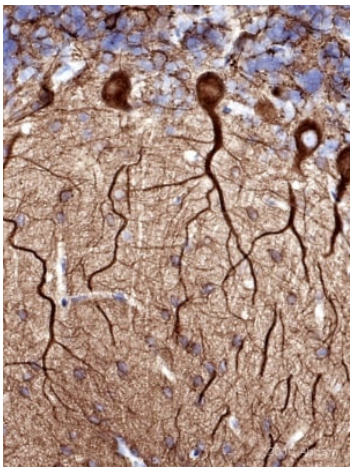
Immunohistochemistical detection of Neuron specific beta III Tubulin using antibody (ab78078) on formaldehyde-fixed paraffin-embedded human medulla oblongata sections. Antigen retrieval step: Heat mediated in citric acid HIER buffer. Permeabilization: No. Blocking step: 1% BSA for 10 mins @ rt°C. Primary antibody dilution 1/250 incubated for 2 hours in TBS/BSA/azide. Secondary antibody: anti Mouse IgGs conjugated to biotin (1/200). This section was cut from an anonymous autopsy P.wax block that is over 20 yrs old, so I would expect variable positivity when compared with more recently sampled tissues (giving higher dilution factors, such as I obtained with fresh mouse/rat CNS blocks.) Transverse-cut axons stain very intensely longitudinal nerve processes and cell bodies are not so heavily stained but are still easily seen.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

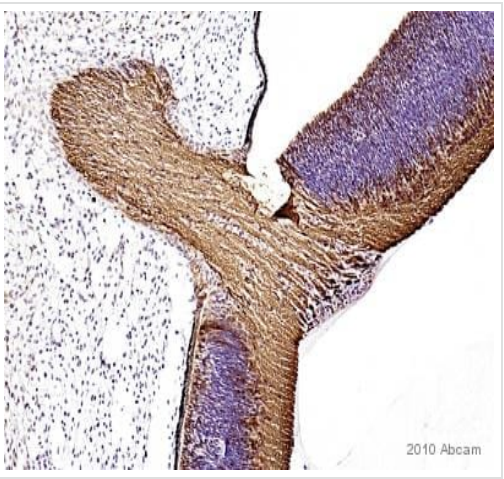
Immunohistochemical detection of Neuron specific beta III Tubulin using ab78078 on formaldehyde fixed dogfish/catshark PNS tissue sections. Antigen retrieval step: heat mediated in citric acid. Blocking: 1% BSA for 10 mins @ rt°C. Primary antibody ab78078 incubated at 1/1250 for 2 hours. Secondary Antibody: anti mouse IgG conjugated to biotin @ 1/200 This is a composite image of neuronal cell bodies and fibres; the upper image uses coloured arrowheads to indicate positive PNS components: cell bodies (black), L/S (green) and T/S (red) nerve fibres of what appear to be three Ganglia. Note that the L/S fibres do not appear as well-stained as the T/S fibres. The lower image shows what appears to be a linear sequence of single nerve cells and their processes within the core of a gill.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

Immunohistochemistical detection of Neuron specific beta III Tubulin antibody using ab78078 on formaldehyde-fixed paraffin-embedded marmoset cerebellum sections. Antigen retrieval step: Heat mediated in Citric acid pH6 buffer. Permeabilization: No. Blocking step: 1% BSA for 10 mins @ rt°C. Primary Antibody used at 1/1000 incubated for 2 hours in TBS/BSA/azide. Secondary Antibody: anti mouse IgG conjugation to biotin (1/200).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

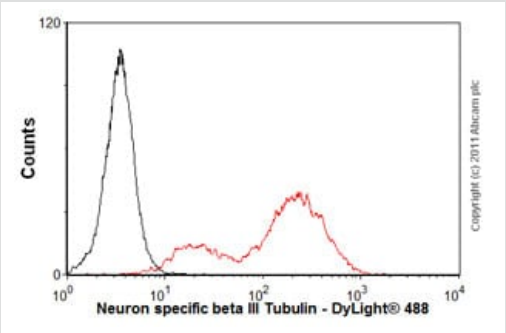
This image is courtesy of an abreview by Carl Hobbs

ab78078 staining beta III Tubulin in Chicken e6: developing eye tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at room temperature; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/1250) for 2 hours. A Biotin-conjugated Goat anti-mouse IgG polyclonal (1/200) was used as the secondary antibody.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

Immunohistochemistical detection of Neuron specific beta III Tubulin antibody [2G10] using ab78078 in formaldehyde-fixed paraffin-embedded quail embryo eye sections. Antigen retrieval step: heat mediated in citric acid pH6 buffer. Blocking step: 1% BSA for 10 mins @ rt°C Primary antibody incubated at 1/1250 for 2 hours in TBS/BSA/azide. Secondary antibody: anti-Mouse IgG conjugated to biotin used at 1/200. In this image, the lining cells of the hyaloid artery are positive. Excellent neural component specificity: even small fibres outside of the cartilage model of the orbit are clearly demonstrated.



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [2G10] - Neuronal Marker (ab78078)

Overlay histogram showing SH-SY5Y stained with ab78078 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab78078, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1](**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol/permeabilized in 0.1% PBS-Tween used under the same conditions.

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