abcam

Product datasheet

Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker ab7751

**** 23 Abreviews 169 References 13 Images

Overview

Product name Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker

Description Mouse monoclonal [TU-20] to beta III Tubulin - Neuronal Marker

Host species Mouse

Specificity Class III beta-tubulin specific for neurons.

Tested applications Suitable for: WB, Flow Cyt (Intra), ICC/IF, IHC-P

Species reactivity Reacts with: Mouse, Human

Predicted to work with: a wide range of other species, Mammals

Immunogen Synthetic peptide corresponding to Human beta III Tubulin aa 400-500 conjugated to keyhole

limpet haemocyanin.

Database link: **Q13509**

Run BLAST with
Run BLAST with

Epitope ESESQGPK (aa 441-448)

Positive control ICC/IF: Neuro-2a, P-19, Primary mouse cortical culture and Induced neurons derived from human

pluripotent cells. WB: Human brain and spinal cord tissue lysate. IHC-P: Human cerebellum

tissue. Flow Cyt (Intra): SH-SY5Y cells.

General notesThe 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

1

Preservative: 0.097% Sodium azide

Constituent: PBS

Purity Protein A purified

Purification notes Purity >95% by SDS-PAGE.

Clonality Monoclonal
Clone number TU-20
Isotype IqG1

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab7751 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
WB	★★★★★(3)	Use a concentration of 1 - 2 µg/ml. We suggest reducing conditions and a 90 minute incubation time.
Flow Cyt (Intra)		1/20 - 1/50. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (8)	1/1000.
IHC-P	★★★★☆ (7)	Use a concentration of 10 μg/ml.

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.

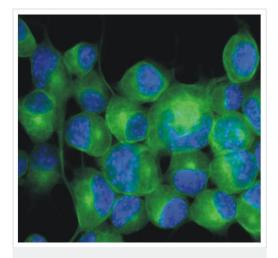
Post-translational modifications

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.

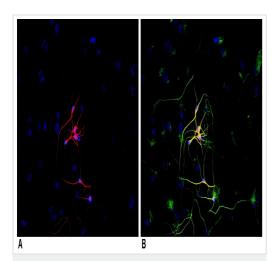
Cellular localization

Cytoplasm > cytoskeleton.

Images



Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751) Immunocytochemistry staining of mouse Neuro2a cells (neuroblast/neuronal and amoeboid stem cells) using 3µg/ml ab7751 (green), nuclear counterstaining with DAPI (blue).

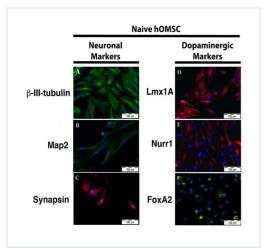


Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

Immunocytochemistry staining of mouse P-19 cells (embryonal carcinoma).

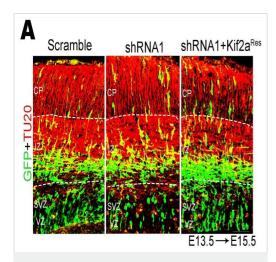
A: Microtubules stained with neuron-specific ab7751 in red.

B: Merged image of co-staining with ab7751 and anti-beta-tubulin.



Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

Ganz et al PLoS One. 2014 Jun 19;9(6):e100445. doi: 10.1371/journal.pone.0100445. eCollection 2014. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody

[TU-20] - Neuronal Marker (ab7751)

Sun et al PLoS One. 2017 Jun 7;12(6):e0179047. doi: 10.1371/journal.pone.0179047. eCollection 2017. Fig 5. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Naive hOMSC express constitutively neuronal and dopaminergic markers.

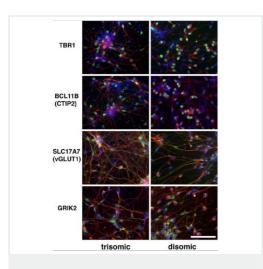
Immunofluorescence of neuronal (A–C) and dopaminergic (D–F) markers in naïve hOMSC, scale bars 100 μ M.

Cells obtained from two donors (24 and 35 years old) were fixed in 4% PFA-PBS and pre-incubated for 60 min in blocking solution (5% goat serum, 1% BSA, 0.05% Triton-X in PBS). Primary antibodies were diluted in the blocking solution and applied overnight at 4°C. The following primary antibodies were used, β -Ill-Tubulin (1 \square 200, ab7751, abcam), Map2 (1 \square 200), synapsin (1 \square 200), tyrosine hydroxylase (1 \square 200), Lmx1A (1 \square 200), Pitx3 (1 \square 200) and Nurr1 (1 \square 200).

Primary antibodies were detected with fluorescent-labeled secondary antibodies Alexa[®]488 and 568 (1□500,) for 1 h at room temperature. Nuclear DNA was stained using 4,6-diamino-2-phenylindole (DAPI) (1□1000).

Kif2a is involved in the differentiation of NSCs/NPCs.

(Panel A) Mouse embryos were electroporated with indicated plasmids (scramble shRNA, Kif2a shRNA1 or Kif2a shRNA1+Kif2a Res) at E13.5, and analyzed at E15.5. GFP (green) represents cells expressing the indicated plasmids; TU20 (ab7751, red) represents immature neurons. Scale bars = $50 \mu m$.

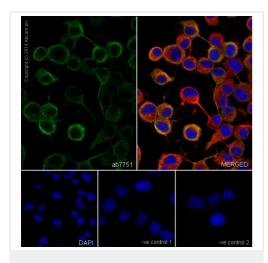


Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

Gonzales et al PLoS One. 2018 Mar 27;13(3):e0194581. doi: 10.1371/journal.pone.0194581. eCollection 2018. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Confirmation of differentiation of iPSCs into cortical neuronal cultures.

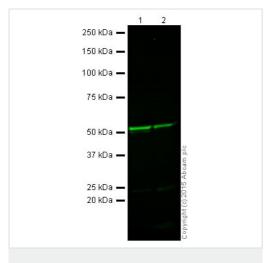
Images taken from clones C2 (trisomic) and C3 -D21 (disomic) 40 days after initiation of the differentiation protocol. Fixed cells on chamber slides were probed with antibodies against the marker proteins listed in the left column. Neuronal marker Beta III tubulin is red (ab7751); DAPI is blue; and Ctip2, TBR1, SLC17A7 (vGLUT1) and GRIK2 are green. Size bar = 20μ .



Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751) ab7751 staining beta III Tubulin in Neuro-2a (Mouse neuroblastoma cell line) cells.

The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab7751 at 1/1000 and **ab6046** at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an AlexaFluor[®]488 Goat anti-Mouse secondary (**ab150117**) at 2 µg/ml (shown in green) and AlexaFluor[®]594 Goat anti-Rabbit secondary (**ab150088**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labeled in blue with DAPI.

Negative controls: 1– Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



Western blot - Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

All lanes : Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751) at 1 µg/ml

Lane 1: Human brain tissue lysate

Lane 2: Human spinal cord tissue lysate

Lysates/proteins at 20 µg per lane.

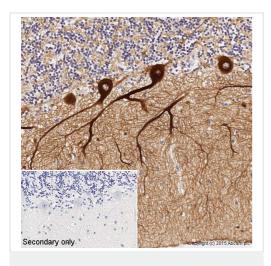
Secondary

All lanes: IRDye® 800CW Goat Anti-Mouse at 1/10000 dilution

Performed under reducing conditions.

Observed band size: 51 kDa

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 LI-COR® blocking buffer before being incubated with ab7751 overnight at 4°C. Antibody binding was detected using the IRDye® 800CW Goat Anti-Mouse secondary at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Odyssey® CLx Imaging System.

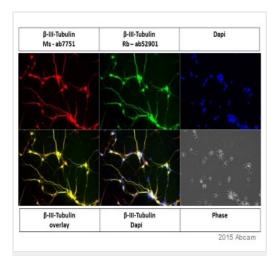


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

IHC image of ab7751 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, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab7751, $5\mu g/ml$, 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 hematoxylin 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.

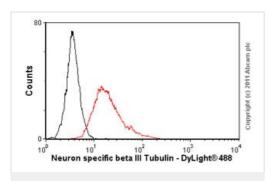


Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

This image is courtesy of an anonymous Abreview

ab7751 staining beta Ill Tubulin in induced neurons derived from human pluripotent cells by ICC (Immunocytochemistry).

Cells were fixed with paraformaldehyde, permeabilized with 0.25% Triton X-100 in PBS and blocked with 1% BSA for 30 minutes at 22°C. Samples were incubated with primary antibody (1/500) for 16 hours at 4°C. An Alexa Fluor[®] 594-conjugated Donkey anti-mouse IgG polyclonal (1/1000) was used as the secondary antibody.

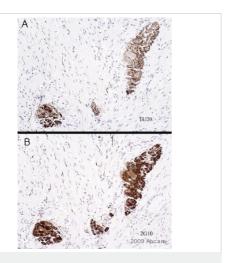


Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

Overlay histogram showing SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells stained with ab7751 (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 (ab7751, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-mouse lgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG1 [ICIGG1] (ab91353, $2\mu g/1x10^6$ 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 (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

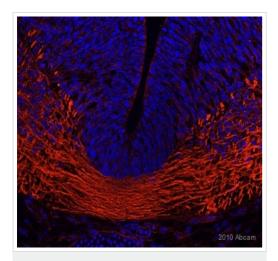


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody
[TU-20] - Neuronal Marker (ab7751)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

ab7751 staining neuron specific beta III Tubulin in human colon tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Tissue underwent formaldehyde fixation before heat mediated antigen retrieval in citric acid pH 6 and then blocking with 1% BSA was performed for 10 minutes at RT. The primary antibody was used at dilution at 1/200 and incubated with sample in TBS/BSA/azide for 2 hours. A biotin conjugated goat polyclonal to mouse IgG was used at dilution at 1/200 as secondary antibody. Images A and B represent staining of the clone TU20 and 2G10 respectively in ganglia of Auerbach's plexus.



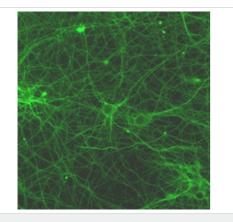
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

Immunohistochemistical detection of neuron specific beta III Tubulin using antibody (ab7751) on whole mouse e12 embryo (formaldehyde fixed/frozen sections).

Permeabilization: No. Blocking step: 1% BSA for 10 mins @ rt°C. Primary Antibody Dilution 1/200; Incubation time 2 hours in TBS/BSA/azide. Secondary antibody: anti Mouse IgG Conjugated to Alexa Fluor[®] 594 (1/1000).

Floorplate region of developing cervical spinal cord (I have not studied this particular region).



ab7751 immunostaining (1/500) neuronal processes in primary mouse cortical culture.

Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [TU-20] - Neuronal Marker (ab7751)

Photo courtesy of QBMCellScience

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