abcam

ab221935

Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP1569Y
Purification technique	Affinity purification Protein A
Concentration	1.042 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human, Rat, Mouse
Dilution info	-
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat

Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Zebrafish
Dilution info	-
Notes	-

Target data

See full target information TUBB3

Function	Tubulin is the major constituent of microtubules, protein filaments consisting of alpha- and beta-tubulin heterodimers (PubMed:34996871, PubMed:38305685, PubMed:38609661). Microtubules grow by the addition of GTP-tubulin dimers to the microtubule end, where a stabilizing cap forms (PubMed:34996871, PubMed:38305685, PubMed:38609661). Below the cap, alpha-beta tubulin heterodimers are in GDP-bound state, owing to GTPase activity of alpha-tubulin (PubMed:34996871, PubMed:38609661). TUBB3 plays a critical role in proper axon guidance and maintenance (PubMed:20074521). Binding of NTN1/Netrin-1 to its receptor UNC5C might cause dissociation of UNC5C from polymerized TUBB3 in microtubules and thereby lead to increased microtubule dynamics and axon repulsion (PubMed:28483977). Plays a role in dorsal root ganglion axon projection towards the spinal cord (PubMed:28483977).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab221935 is the carrier-free version of ab52623.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For

details on our patents, please refer to RabMAb® patents.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Beta III tubulin often referred to as βIII tubulin or beta-tubulin 3 is a microtubule element involved in the cellular cytoskeleton structure. This protein with a molecular mass of approximately 55 kDa plays an important role in the development and maintenance of neuron structures. It is most prominently expressed in neurons within the central and peripheral nervous systems. In these cells beta III tubulin contributes to microtubule polymerization facilitating the formation of the intricate network necessary for cell shape intracellular transport and division.
Biological function summary	Beta III tubulin acts in stabilizing microtubules which are essential for proper neuronal function. It exists as part of the tubulin dimer complex partnering with alpha-tubulin to form the building blocks of microtubules. This particular isotype is often used as a marker for neuronal differentiation due to its specific expression pattern in neural tissues. Its regulation and expression levels are important for neurogenesis and maintaining neuronal plasticity.
Pathways	Beta III tubulin plays a role in neuron-specific intracellular transport and signaling pathways like the Rho GTPase and MAPK signaling pathways. These pathways are involved in cell cycle regulation and apoptotic processes. Relationships with other proteins such as kinesins and dyneins are important in these pathways influencing intracellular transport and signaling through binding and moving along the microtubule tracks created by beta-tubulin isotypes including beta III tubulin.
Associated diseases and disorders	Abnormal beta III tubulin expression has been associated with cancer particularly in tumors originating from neuronal lineage such as glioblastomas. Overexpression or mutations can contribute to chemoresistance complicating treatment for certain types of cancer. Beta III tubulin is also linked to neurodevelopmental disorders as it affects proper neural network formation and stability. Its relationship with tumor protein p53 is noted in cancer pathways as p53 can influence beta III tubulin expression impacting cellular proliferation and apoptosis in oncogenic processes.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

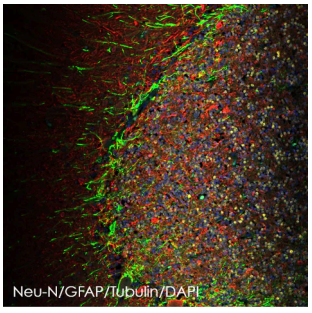
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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12 product images



Multiplex immunohistochemistry - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Multiplex immunohistochemistry staining using rabbit Anti-beta III Tubulin antibody

This data was developed using the same antibody clone in a different buffer formulation (ab52623).
Fluorescence multiplex immunohistochemical analysis of human cerebellum tissue (formalin-fixed paraffin-embedded section).

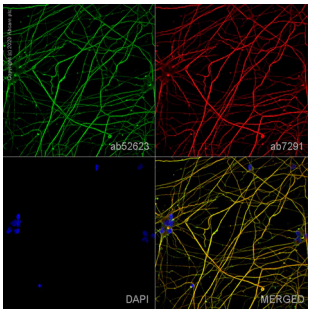
Merged staining of Neu-N (ab177487; yellow; Opal™570), anti-beta III Tubulin (ab52623; red; Opal™690) and anti-GFAP (ab68428; green; Opal™520).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ kit.

The section was incubated in three rounds of staining with ab177487 (1/1000 dilution), ab52623 (1/200 dilution) and ab68428 (1/250 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (pH 6.0) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

DAPI (blue) was used as a nuclear counterstain.



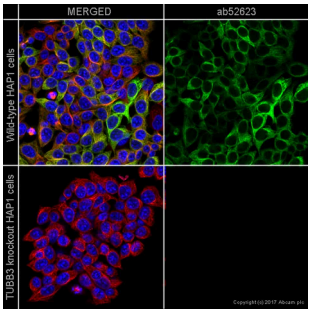
Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-beta III Tubulin antibody

This ICC/IF data was generated using the same anti-beta III tubulin antibody clone, EP1569Y, in a different buffer formulation (ab52623).
Immunofluorescence staining of beta III Tubulin using ab52623 in ioGlutamatergic Neurons (Human iPSC-Derived Glutamatergic Neurons, ab259259), which were differentiated for 11 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins 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 ab52623 at 0.1 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with ab150081, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150120, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown.

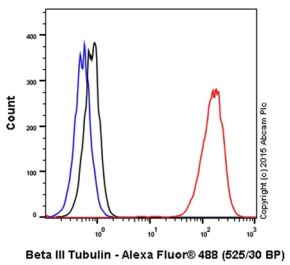


Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-beta III Tubulin antibody

This ICC/IF data was generated using the same anti-beta III tubulin antibody clone, EP1569Y, in a different buffer formulation (cat# ab52623).
ab52623 staining beta III Tubulin in wild-type HAP1 cells (top panel) and TUBB3 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% 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 with ab52623 at 1/500 dilution and ab195889 at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

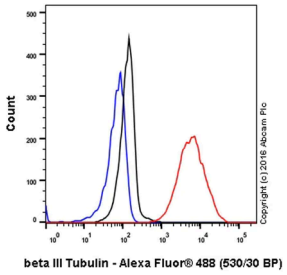
Beta III Tubulin Flow Cytometry (Intracellular) staining using rabbit Anti-beta III Tubulin antibody

Overlay histogram showing U-87MG cells stained with ab52623 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 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 (ab52623, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at

1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730, 0.1µg/1x10^6) used under the same conditions. Unlabelled sample (blue line) was also used as a control.
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in U-87MG cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

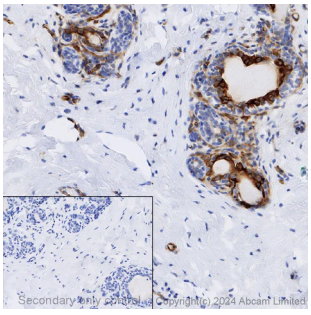
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52623).



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Flow Cytometry (Intracellular) staining using rabbit Anti-beta III Tubulin antibody

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling beta III Tubulin with ab52623 at 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52623).

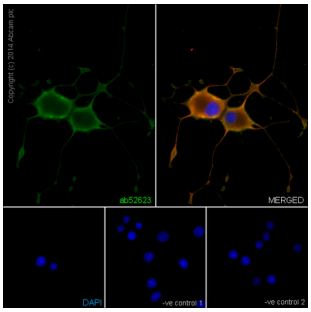


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52623).
Immunohistochemical analysis of formalin fixed paraffin embedded human breast carcinoma labelling beta III Tubulin with ab52623 at a concentration of 0.5 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5 for 32mins.

ab52623 anti-beta III Tubulin [EP1569Y] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

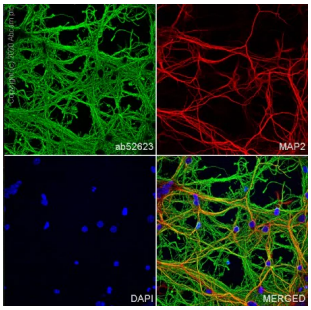
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-beta III Tubulin antibody

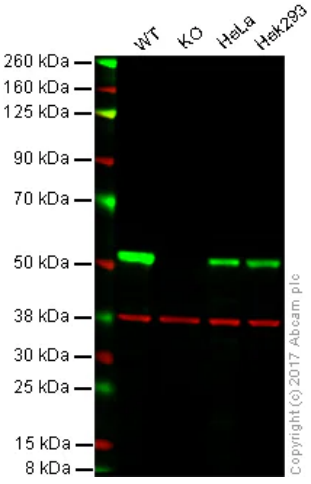
ab52623 staining beta III Tubulin in NGF-differentiated PC12 cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab52623 at 5µg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an AlexaFluor®488 Goat anti-Rabbit secondary (ab150081) at 2 µg/ml (shown in green) and AlexaFluor®594 Goat anti-Mouse secondary (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52623).



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Immunocytochemistry/ Immunofluorescence staining of Mouse primary neurons using rabbit Anti-beta III Tubulin antibody

This image was produced using ab52623, the same clone but in a different formulation. Immunofluorescent analysis of 4% Paraformaldehyde fixed 0.1% Triton X-100 permeabilized transfected mouse primary neurons labelling beta III Tubulin with ab52623 at 1/500 followed by ab150077 Goat Anti-Rabbit (Alexa Fluor® 488) preadsorbed antibody as secondary at 1/1000 (2 µg/ml) dilution. Positive staining in mouse primary neurons (shown in green). ab11267 Anti-MAP2 mouse monoclonal antibody at 1/500 (5µg/ml) was used as a counterstain followed by ab150120 AlexaFluor® 594 Goat anti-Mouse secondary (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Western blot staining using rabbit Anti-beta III Tubulin antibody

This WB data was generated using the same anti-beta III tubulin antibody clone, EP1569Y, in a different buffer formulation (cat# ab52623).
Lanes 1 - 4: Merged signal (red and green). Green - ab52623 observed at 52 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab52623 was shown to specifically react with beta III Tubulin in wild-type cells as signal was lost in beta III Tubulin knockout HAP1 cells. Wild-type and beta III Tubulin knockout samples were subjected to SDS-PAGE. ab52623 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:

Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) at 1/1000 dilution

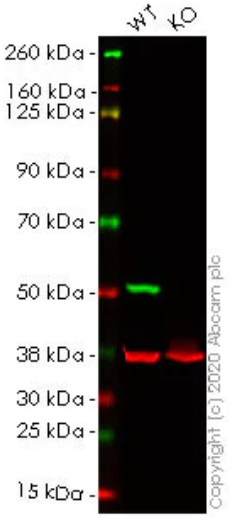
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
Beta III Tubulin knockout HAP1 whole cell lysate at 20 µg

Lane 3:
HeLa whole cell lysate at 20 µg

Lane 4:
HEK293 whole cell lysate at 20 µg

Predicted band size: 50 kDa



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Western blot staining using rabbit Anti-beta III Tubulin antibody

This data was developed using the same antibody clone in a different buffer formulation (ab52623).
Lanes 1- 2: Merged signal (red and green). Green - ab52623 observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab52623 was shown to react with beta III tubulin in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255358 (knockout cell lysate ab263857) was used. Wild-type HeLa and TUBB3 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab52623 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) at 1/1000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

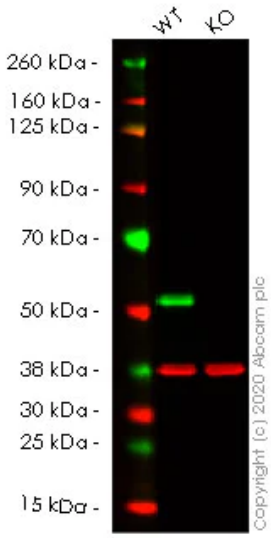
Lane 2:
TUBB3 knockout HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human TUBB3 (beta III Tubulin) knockout HeLa cell line (ab255358)

Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 50 kDa



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Western blot staining using rabbit Anti-beta III Tubulin antibody

This data was developed using the same antibody clone in a different buffer formulation (ab52623).
Lanes 1- 4: Merged signal (red and green). Green - ab52623 observed at 52 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab52623 was shown to react with beta III Tubulin in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line ab266900 (knockout cell lysate ab257070) was used. Wild-type HCT116 and TUBB3 knockout HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab52623 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) at 1/1000 dilution

Lane 1:
Wild-type HCT116 cell lysate at 20 µg

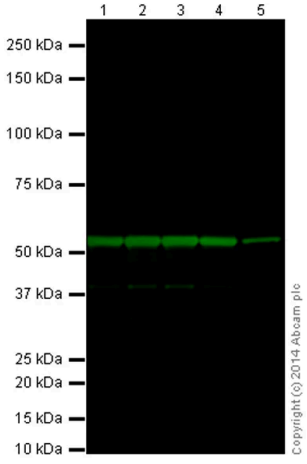
Lane 2:
TUBB3 knockout HCT116 cell lysate at 20 µg

Lane 2:
Western blot - Human TUBB3 (beta III Tubulin) knockout HCT116 cell line (ab266900)

Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 52 kDa



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - BSA and Azide free (ab221935)

Beta III Tubulin Western blot staining using rabbit Anti-beta III Tubulin antibody

This data was developed using the same antibody clone in a different buffer formulation (ab52623).
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 Licor blocking buffer before being incubated with ab52623 overnight at 4°C.

Antibody binding was detected using ab175781 at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

All lanes:
Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) at 1/1000 dilution

Lane 1:

Brain (Mouse) Tissue Lysate at 20 µg

Lane 2:
Brain (Rat) Tissue Lysate at 20 µg

Lane 3:
Spinal Cord (Mouse) Tissue Lysate at 20 µg

Lane 4:
Spinal Cord (Rat) Tissue Lysate at 20 µg

Lane 5:
PC-12 (Rat adrenal pheochromocytoma cell line) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) at 1/10000 dilution

Predicted band size: 50 kDa

Observed band size: 52 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.