abcam

Product datasheet

Anti-beta III Tubulin antibody [EPR19591] ab215037

KO VALIDATED Recombinant RabMAb

4 References 18 Images

Overview

Product name	Anti-beta III Tubulin antibody [EPR19591]
Description	Rabbit monoclonal [EPR19591] to beta III Tubulin
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human cerebellum, breast cancer, fetal kidney and fetal brain lysates; U-87 MG, SH-SY5Y, HEK-293, C6, PC-12, HCT116, Hap1 and HeLa whole cell lysates; Mouse brain lysate; Rat brain and heart lysates. IHC-P: Human cerebral cortex, glioma, cholangiocarcinoma and lung adenocarcinoma tissues; Mouse and rat cerebral cortex tissues. ICC/IF: SH-SY5Y and U-87 MG cells. Flow Cyt (intra): U-87 MG cells, HAP1 cells. IP: U-87 MG whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR19591
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab215037 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		1/2000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).
ICC/IF		1/500.
IP		1/30.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/200.

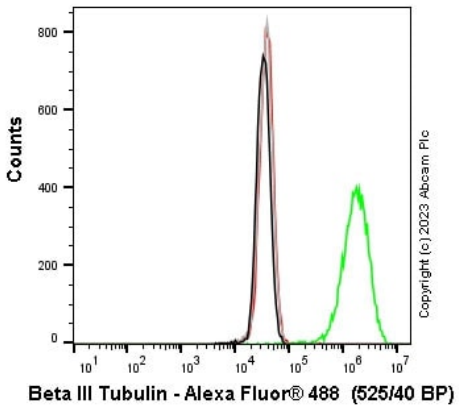
Target

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



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

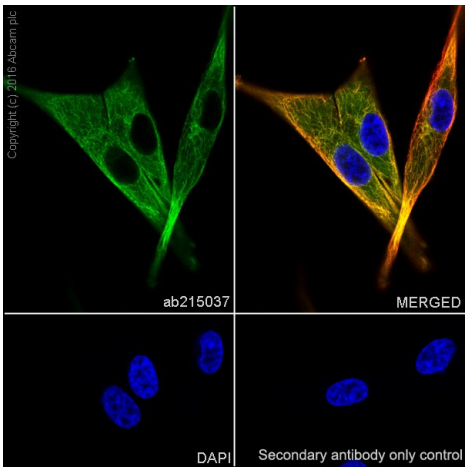
Flow cytometry overlay histogram showing wild-type Hap1 (green line) and TUB3 knockout Hap1 stained with ab215037 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab215037) (1x 10^6 in 100µl at 0.2 µg/ml (1/10400)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, TUB3 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Hap1 Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

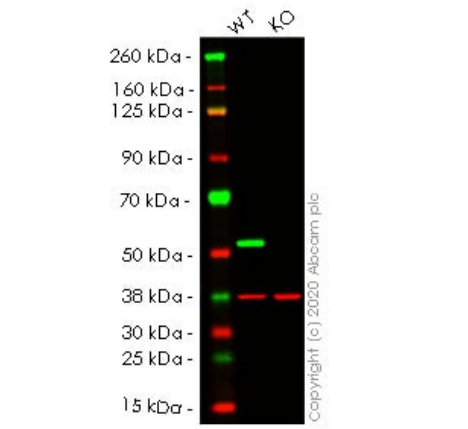
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) cells labeling beta III Tubulin with ab215037 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on U-87 MG cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Western blot - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

All lanes : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/2000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : TUBB3 knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

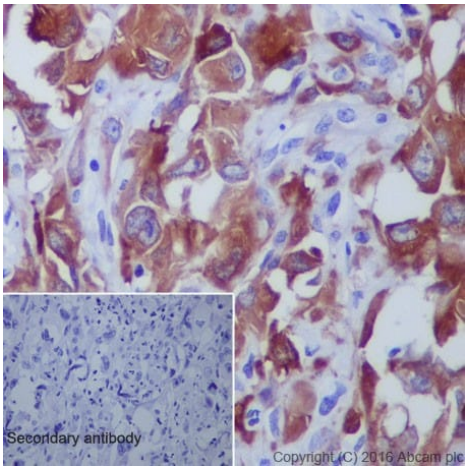
Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 52 kDa

Lanes 1- 4: Merged signal (red and green). Green - ab215037 observed at 52 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab215037 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. ab215037 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 2000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

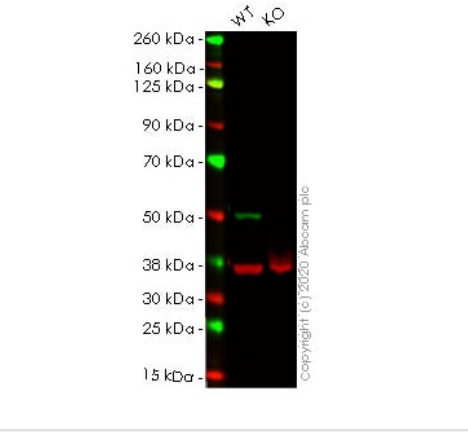
Immunohistochemical analysis of paraffin-embedded human cholangiocarcinoma tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Cytoplasmic staining on human cholangiocarcinoma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

All lanes : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : TUBB3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

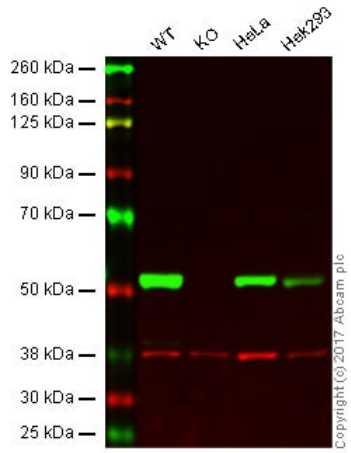
Performed under reducing conditions.

Predicted band size: 50 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab215037 observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab215037 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. ab215037 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**)

overnight at 4°C at a 1 in 2000 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.



Western blot - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

All lanes : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/2000 dilution

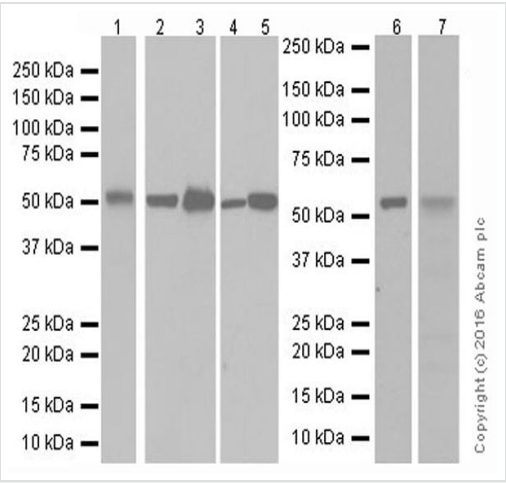
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : TUBB3 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : HEK293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 50 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab215037 observed at 50 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab215037 was shown to specifically react with TUBB3 in wild-type HAP1 cells as signal was lost in TUBB3 knockout cells. Wild-type and TUBB3 knockout samples were subjected to SDS-PAGE. ab215037 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/2000 dilution and 1/20,000 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 1/20,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

Lanes 1-5 : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/2000 dilution
Lanes 6-7 : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/10000 dilution

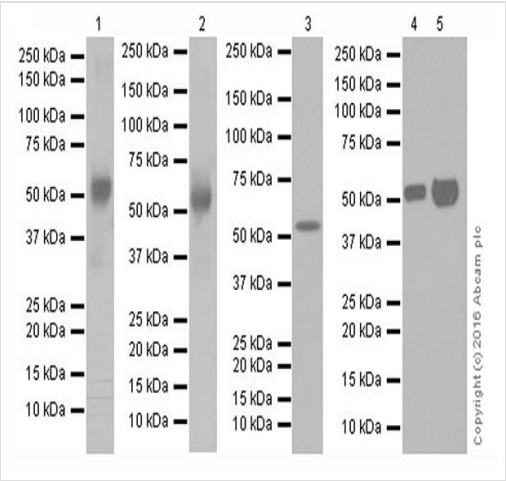
Lane 1 : Human cerebellum lysate at 20 µg
Lane 2 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate at 20 µg
Lane 3 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate at 20 µg
Lane 4 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 20 µg
Lane 5 : Human breast cancer lysate at 20 µg
Lane 6 : Human fetal kidney lysate at 10 µg
Lane 7 : Human fetal brain lysate at 10 µg

Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/7: 1 second; Lane 2/3: 5 seconds; Lane 4/5: 3 minutes; Lane 6:30 seconds.



Western blot - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

Lanes 1-2 : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/2000 dilution
Lane 3 : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/5000 dilution
Lanes 4-5 : Anti-beta III Tubulin antibody [EPR19591] (ab215037) at 1/10000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Rat brain lysate
Lane 3 : Rat heart lysate
Lane 4 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 5 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

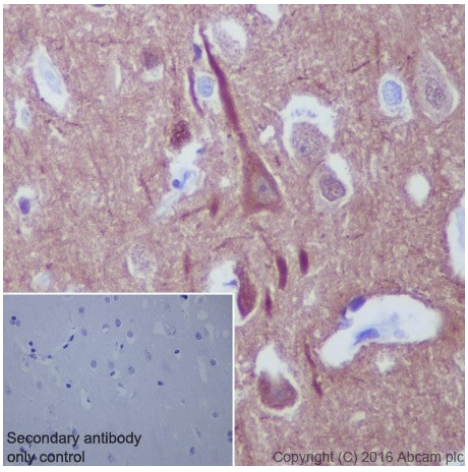
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 1 second; Lane 3: 3 minutes; Lane 4/5: 30 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

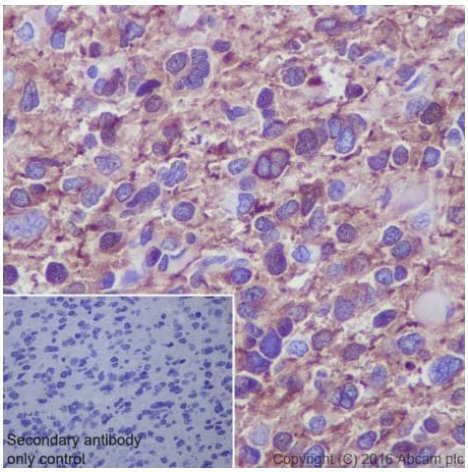
Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Cytoplasmic staining on human cerebral cortex is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

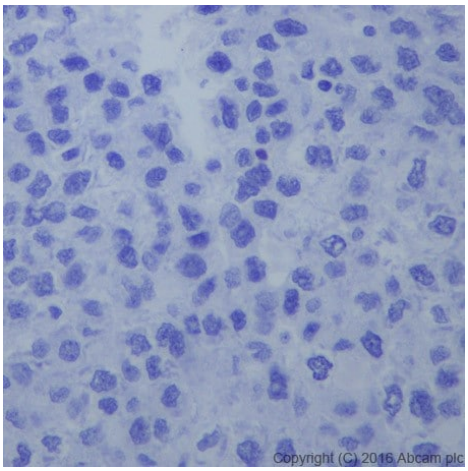
Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Cytoplasmic staining on human glioma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



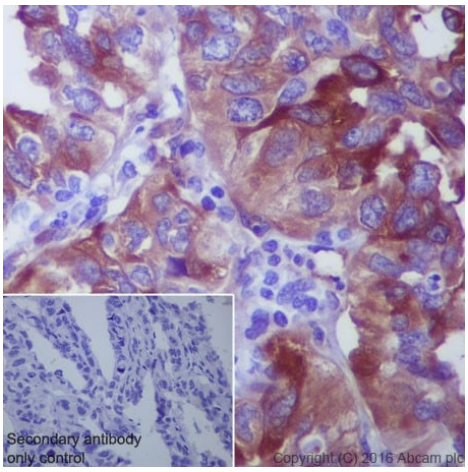
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Negative staining on human hepatocellular carcinoma. (PMID: 25039376).

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

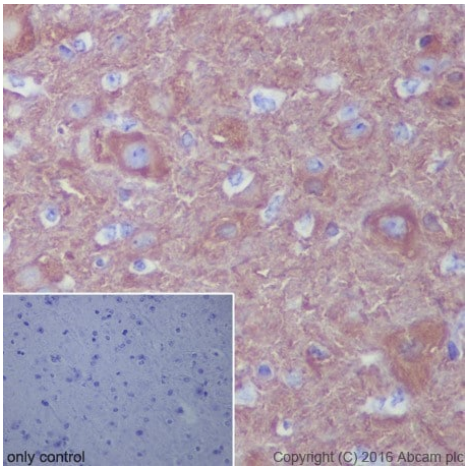
Immunohistochemical analysis of paraffin-embedded human lung adenocarcinoma tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Cytoplasmic staining on human lung adenocarcinoma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

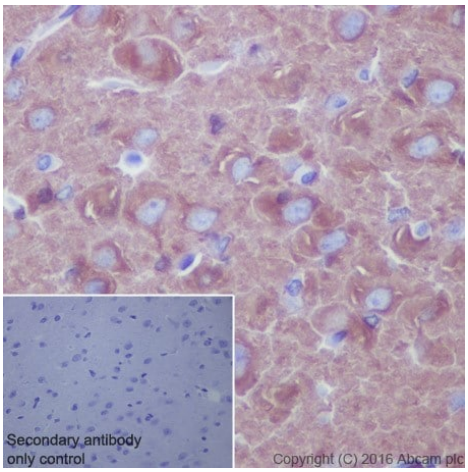
Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Cytoplasmic staining on mouse cerebral cortex is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

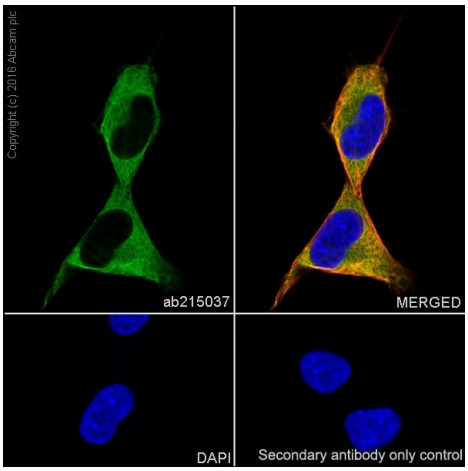
Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling beta III Tubulin with ab215037 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Cytoplasmic staining on rat cerebral cortex is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

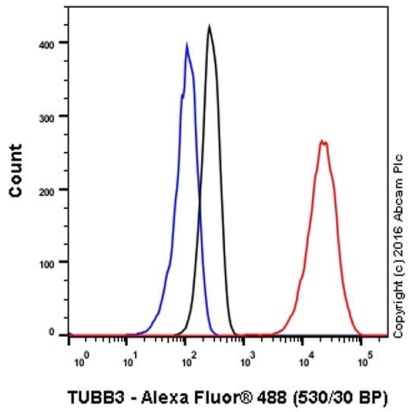
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells labeling beta III Tubulin with ab215037 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on SH-SY5Y cell line.

The nuclear counterstain is DAPI (blue).

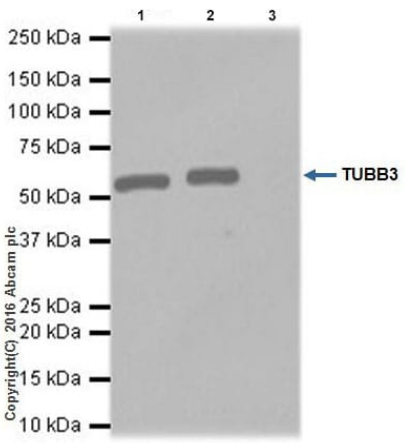
Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) cells labeling beta III Tubulin with ab215037 at 1/200 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-beta III Tubulin antibody [EPR19591] (ab215037)

beta III Tubulin was immunoprecipitated from 0.35 mg of U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate with ab215037 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab215037 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution

Lane 1: U-87 MG whole cell lysate 10µg (Input).

Lane 2: ab215037 IP in U-87 MG whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab215037 in U-87 MG whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

Anti-beta III Tubulin antibody [EPR19591] (ab215037)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors