abcam

Product datasheet

Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker ab52623

敲除验证 重组 RabMAb

★★★★★ 6 Abreviews 45 References 14 图像

概述

产品名称	Anti-beta III Tubulin抗体[EP1569Y] - Neuronal Marker
描述	兔单克隆抗体[EP1569Y] to beta III Tubulin - Neuronal Marker
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), mIHC, ICC/IF, WB, IHC-P
种属反应性	**与反应:** Mouse, Rat, Human **预测可用于:** Zebrafish
免疫原	Synthetic peptide within Human beta III Tubulin aa 400 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **Q13509**
阳性对照	ICC/IF: PC-12, HAP1 and HeLa cells. WB: HAP1 cell lysate. HeLa, HCT116, PC-12 and HEK-293 whole cell lysate. Mouse and rat brain and spinal cord tissue lysate. IHC-P: Human breast carcinoma tissue. mIHC: Human cerebellum tissue Flow Cyt (intra): U-87 MG and HeLa cells. IHC-Fr: Zebrafish retina sections.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture

	supernatant
纯度	Protein A purified
克隆	单克隆
克隆编号	EP1569Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab52623于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/10 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
mIHC		1/1000. Perform Sodium citrate antigen retrieval (pH 6.0) between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.
ICC/IF	★★★★☆ (1)	1/500.
WB	★★★★★ (3)	1/1000 - 1/10000. Detects a band of approximately 52 kDa.
IHC-P	★★★★★ (1)	1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

靶标

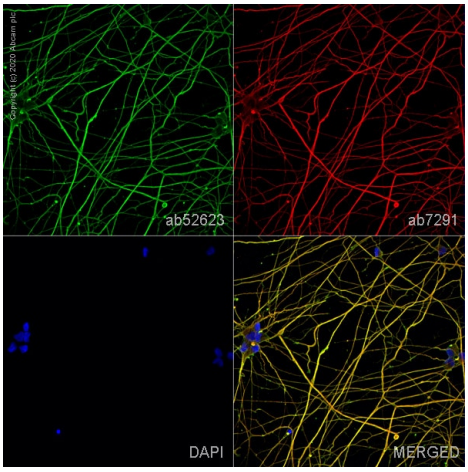
功能 Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. TUBB3 plays a critical role in proper axon guidance and mantainance.

组织特异性 Expression is primarily restricted to central and peripheral nervous system.

疾病相关 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.
序列相似性	Belongs to the tubulin family.
结构域	The highly acidic C-terminal region may bind cations such as calcium.
翻译后修饰	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.
细胞定位	Cytoplasm > cytoskeleton.

图片

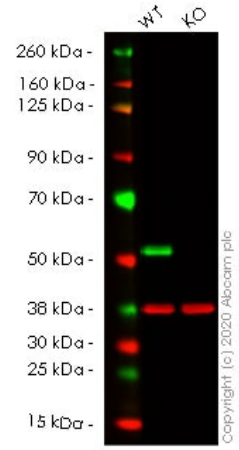


Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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.



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

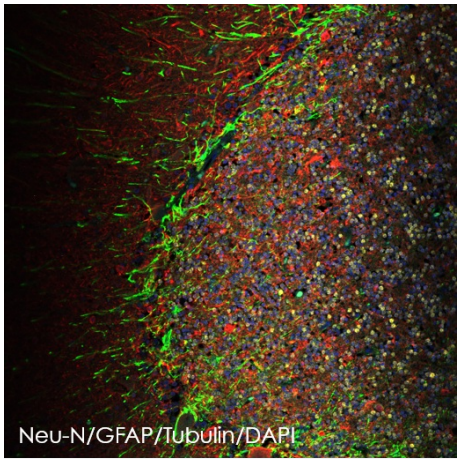
All lanes : Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) at 1/1000 dilution

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.

Lanes 1- 2: Merged signal (red and green). Green - ab52623 observed at 52 kDa. Red - loading control **ab8245** observed at 37 kDa.
ab52623 Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker was shown to specifically 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 and beta III Tubulin knockout samples 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**) were incubated overnight at 4°C at 1 in 1000 and 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.



Multiplex immunohistochemistry - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

Fluorescence multiplex immunohistochemical analysis of human cerebellum tissue (formalin-fixed paraffin-embedded section).

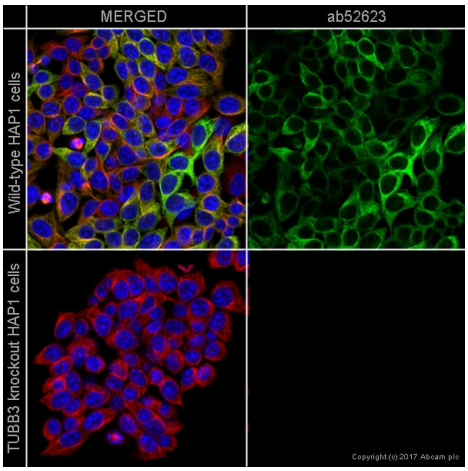
Merged staining of Neu-N (**ab177487**; yellow; Opal™570), anti-beta III Tubulin (ab52623; red; Opal™690) and anti-GFAP (**ab68428**; green; Opal™520).

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 counter stain.



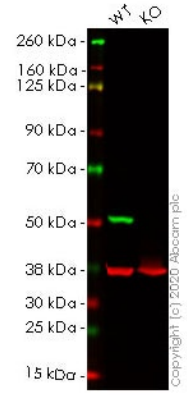
Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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 for 5 minutes, 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 1 hour. 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 1 hour with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green).

Nuclear DNA was labeled in blue with DAPI.

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



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

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

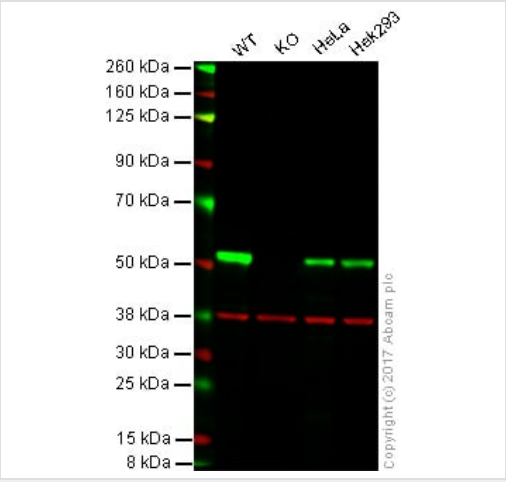
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 50 kDa

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.



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

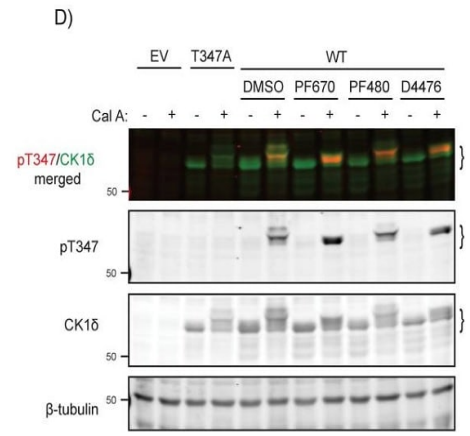
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Beta III Tubulin knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : HEK293 whole cell lysate

Lysates/proteins at 20 µg per lane.

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.

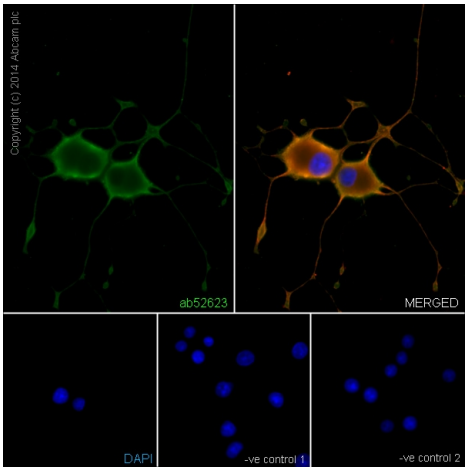
Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

In vivo, CK1δ T347 is phosphorylated in *trans*.

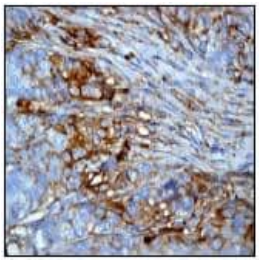
D) Phosphorylation of CK1δ at T347 is not dependent on CK1 activity. HEK-293 cells over-expressing wild-type CK1δ were treated with CK1 inhibitors PF670462 (PF670, 1 µM), PF4800567 (PF480, 1 µM), or D4476 (30 µM) for 3 hours, followed by addition for 30 min of either DMSO or 40 nM Calyculin A. Cell lysates were immunoblotted for anti-pT347 or anti-Myc antibodies. CK1 inhibitors block the autophosphorylation-induced mobility shift but do not decrease phosphorylation of T347. Myc-tagged CK1δ indicated by curly brackets. ab52623 lower panel.



Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

ab52623 staining beta III Tubulin in NGF-differentiated PC-12 (Rat adrenal gland pheochromocytoma cell line) cells.

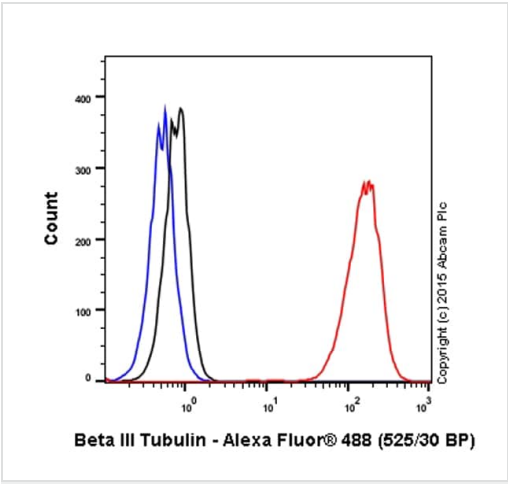
The cells were fixed with 100% methanol (5 minutes) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab52623 at 5 µg/ml and **ab7291** at 1 µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1 hour 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 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

ab52623, at a 1/50 dilution, staining class III beta Tubulin in human breast carcinoma tissue using Immunohistochemistry, Paraffin embedded tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



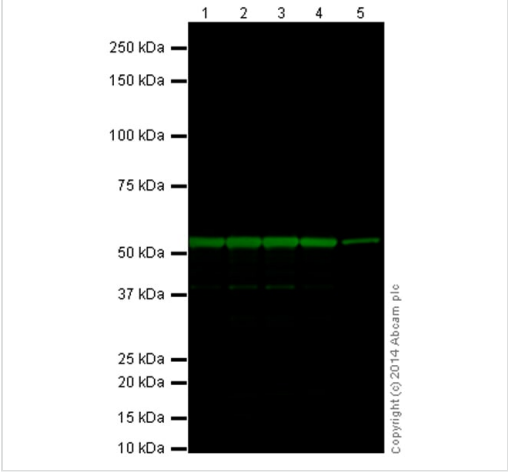
Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

Overlay histogram showing U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) 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 Fluorr®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**, 0.1 µg/1x10^6) used under the same conditions. Unlabeled 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-87 MG cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

Lane 1 : Brain (Mouse) Tissue Lysate
Lane 2 : Brain (Rat) Tissue Lysate
Lane 3 : Spinal Cord (Mouse) Tissue Lysate
Lane 4 : Spinal Cord (Rat) Tissue Lysate
Lane 5 : PC-12 (Rat adrenal pheochromocytoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

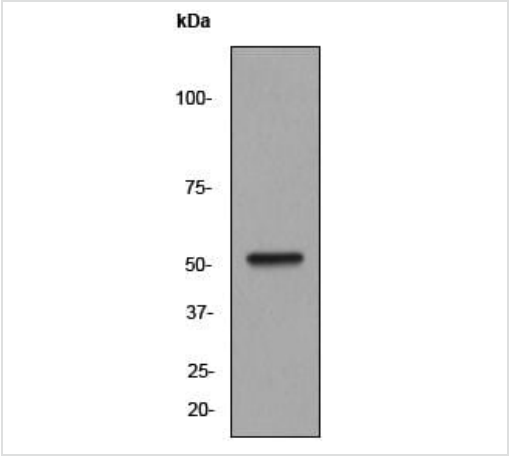
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (**ab175781**) at 1/10000 dilution

Observed band size: 52 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 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.



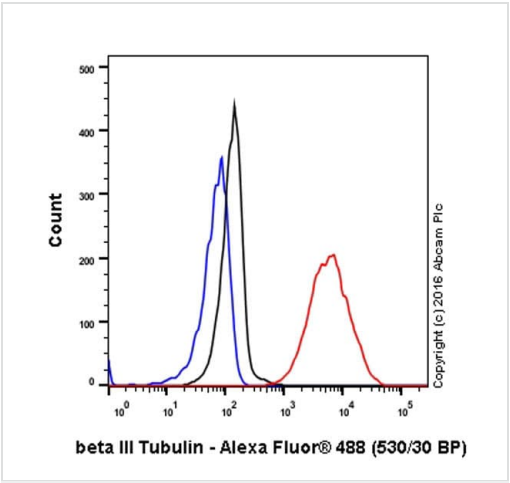
Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) at 1/10000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

Secondary

goat anti-rabbit HRP at 1/2000 dilution

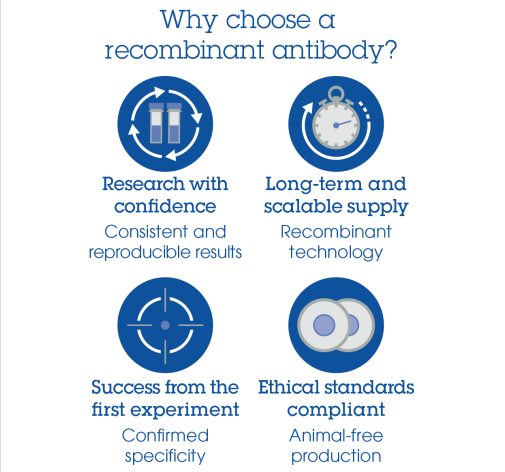
Observed band size: 52 kDa



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

Intracellular Flow Cytometry analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling beta III Tubulin with ab52623 at 1/20 (red).

Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. An Alexa Fluorr®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabeled control, cells without incubation with primary and secondary antibodies.



Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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