abcam

ab52623

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

Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) is a rabbit monoclonal antibody detecting beta III Tubulin in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF, mIHC**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 50 publications

Lab Essentials | Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Tissue culture supernatant, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | EP1569Y |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.104 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

### IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 - 1/100 |
| **Notes** | Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |

### Expected

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

### Predicted

| | |
|---|---|
| **Species** | Zebrafish |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Zebrafish |
| **Dilution info** | - |
| **Notes** | - |

## mIHC

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | 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. |

### Expected

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

### Predicted

| | |
|---|---|
| **Species** | Zebrafish |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/500 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/500 |

| | |
|---|---|
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/500 |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Zebrafish |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/10 - 1/1000 |
| **Notes** | ab172730 - 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** | Conditional Ambient |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**Product Specifications**
Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, WB, mIHC in human, mouse, rat samples.
Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) specifically detects beta III Tubulin (UniProt ID: Q13509; Molecular weight: 50kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

**Quality and Validation**
Abcam's high quality manufacturing and validation processes ensure Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
The specificity of Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) has been confirmed by testing in knockout samples.
Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) has been cited over 55 times in peer reviewed journals and is trusted by the scientific community.
Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623) has 6 independent reviews from customers.

**Related Products**
Antibody clone EP1569Y is also available pre-conjugated to a variety of labels for your convenience - HRP, Alexa

Fluor® 647, Biotin, Alexa Fluor® 594, Alexa Fluor® 555, APC, FITC, PE, Alexa Fluor® 488 (ab190574, ab190575, ab195903, ab201740, ab202519, ab224977, ab224978, ab224979, ab283728).

**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.

**What are the advantages of a recombinant monoclonal antibody?**
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 batch production

For more information, read more on recombinant antibodies.

## 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**

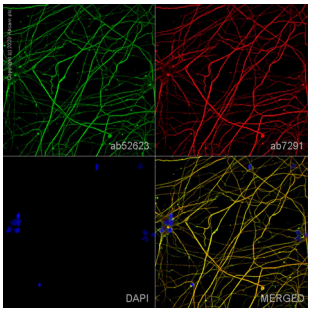
We do not recommend this combination. It is not covered by our product promise.

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.

## 19 product images

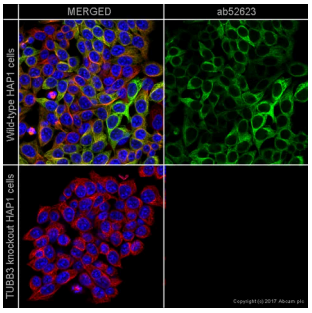



### Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

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.



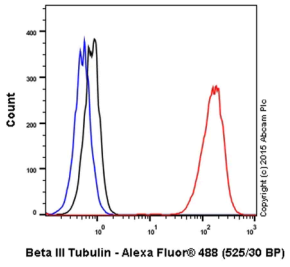

## Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

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).



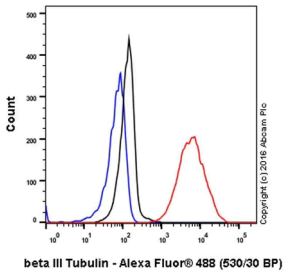

## Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

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/1x$10^6$) 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.

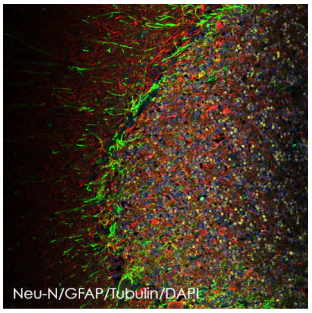
This antibody gave a positive signal in U-87 MG cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.




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

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

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.




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

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

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).

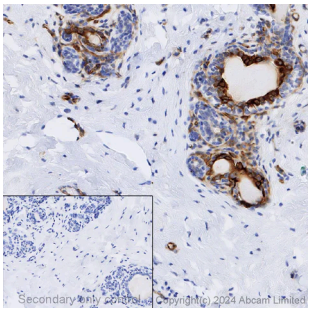
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ kit.

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.

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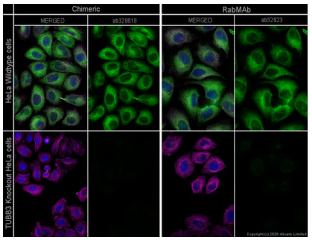

## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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).

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

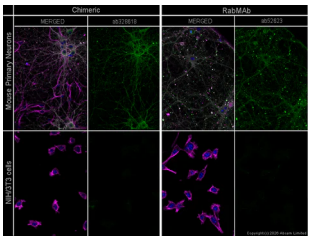
Beta III Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-beta III Tubulin antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized TUBB3 KO HeLa (ED010336) and wildtype HeLa cells labelling beta III Tubulin with ab328618 at 5.0 µg/ml dilution, followed by ab150173 Goat Anti-Chicken IgY H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Left four panels: Chicken chimeric ab328618 [EP1569Y] staining TUBB3 in wildtype HeLa cells (positive) and TUBB3 knockout HeLa cells (negative). The cells were fixed with 4% paraformaldehyde (10 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 for 2 hours at room temperature with ab328618 at 5 µg/ml (shown in green) and ab7291, Anti-alpha Tubulin antibody [DM1A] - Loading Control (shown in pseudocolour magenta). Cells were then incubated with ab150173, Goat Anti-Chicken IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution and ab150120, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution. Nuclear DNA was labeled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Revvity) and a maximum intensity projection of confocal sections is shown.

Right four panels: Side-by-side testing with the original rabbit version of the same clone, ab52623 (Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker).



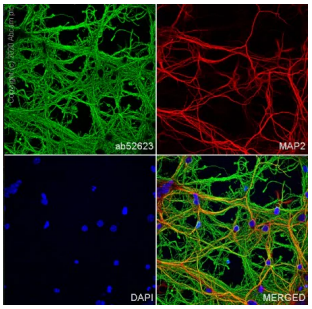

## Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized Mouse Primary Neurons and NIH/3T3 cells labelling beta III Tubulin with ab328618 at 1.0 µg/ml dilution, followed by ab150173 Goat Anti-Chicken IgY H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Left four panels: Chicken chimeric ab328618 [EP1569Y] staining TUBB3 in primary mouse neurons (positive) and NIH/3T3 cells (negative). 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 for 2 hours at room temperature with ab328618 at 1 µg/ml (shown in green) and ab7291, Anti-alpha Tubulin antibody [DM1A] - Loading Control (shown in pseudocolour magenta). Cells were then incubated with ab150173, Goat Anti-Chicken IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution and ab150120, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution. Nuclear DNA was labeled 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, Revvity) and a maximum intensity projection of confocal sections is shown.

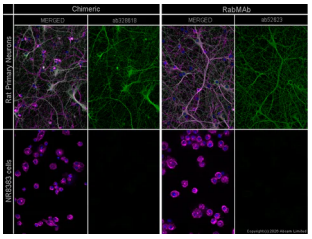
Right four panels: Side-by-side testing with the original rabbit version of the same clone, ab52623 (Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker).




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

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

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).



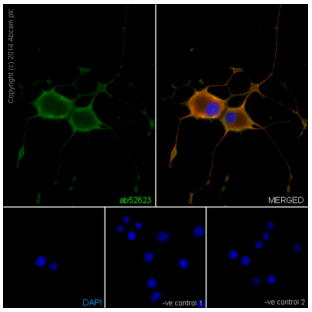

## Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized Primary rat neurons and NR8383 cells labelling beta III Tubulin with ab328618 at 5.0 µg/ml dilution, followed by ab150173 Goat Anti-Chicken IgY H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Left four panels: Chicken chimeric ab328618 [EP1569Y] staining TUBB3 in primary rat neurons (positive) and NR8383 cells (negative). 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 for 2 hours at room temperature with ab328618 at 5 µg/ml (shown in green) and ab7291, Anti-alpha Tubulin antibody [DM1A] - Loading Control (shown in pseudocolour magenta). Cells were then incubated with ab150173, Goat Anti-Chicken IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution and ab150120, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution. Nuclear DNA was labeled 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, Revvity) and a maximum intensity projection of confocal sections is shown.

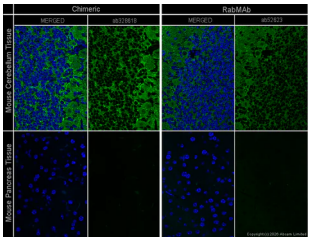
Right four panels: Side-by-side testing with the original rabbit version of the same clone, ab52623 (Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker).




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

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

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)

Beta III Tubulin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-beta III Tubulin antibody

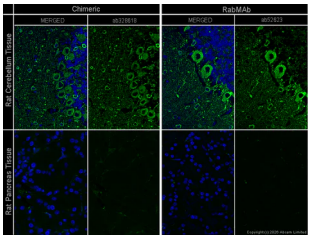
Immunohistochemical analysis of paraffin-embedded Mouse Cerebellum Tissue and Mouse Pancreas Tissue labeling beta III Tubulin with ab328618 at 1 µg/ml dilution, followed by ab150173 Goat Anti-Chicken IgY H&L (Alexa Fluor® 488) preadsorbed at 1:1000 dilution.

Left four panels: Chicken chimeric ab328618 staining TUBB3 [EP1569Y] in sections of formalin-fixed paraffin-embedded mouse cerebellum (positive) and mouse pancreas (negative).Performed on a Leica BOND.

The section was pre-treated using heat mediated antigen retrieval with EDTA (pH9.0) for 40 minutes. The section was then incubated at room temperature for 1 hour with ab328618 at 1/500 dilution, and then incubated for 1 hour with ab150173, Goat Anti-Chicken IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Dako Fluorescence Mounting Medium®. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

Right four panels: Side-by-side testing with the original rabbit version of the same clone, ab52623 (Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker).




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

Beta III Tubulin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-beta III Tubulin antibody

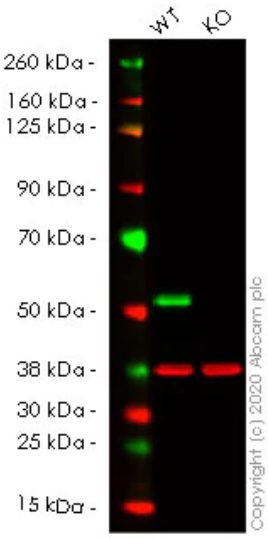
Immunohistochemical analysis of paraffin-embedded Rat Cerebellum Tissue and Rat Pancreas Tissue tissue labeling beta III Tubulin with ab328618 at 1 µg/ml dilution, followed by ab150173 Goat Anti-Chicken IgY H&L (Alexa Fluor® 488) preadsorbed at 1:1000 dilution.

Left four panels: Chicken chimeric ab328618 staining TUBB3 [EP1569Y] in sections of formalin-fixed paraffin-embedded rat cerebellum (positive) and rat pancreas (negative).Performed on a Leica BOND.

The section was pre-treated using heat mediated antigen retrieval with EDTA (pH9.0) for 40 minutes. The section was then incubated at room temperature for 1 hour with ab328618 at 1/500 dilution, and then incubated for 1 hour with ab150173, Goat Anti-Chicken IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Dako Fluorescence Mounting Medium®. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

Right four panels: Side-by-side testing with the original rabbit version of the same clone, ab52623 (Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker).




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

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

**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.
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

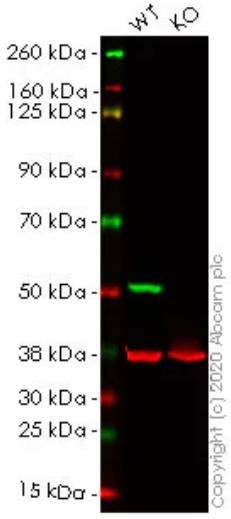
Lane 2:
TUBB3 knockout HCT116 cell lysate at 20 µg

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] - Neuronal Marker (ab52623)

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

**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

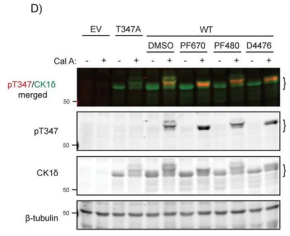
Lane 2:
Western blot - Human TUBB3 (beta III Tubulin) knockout HeLa cell line (ab255358)

Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 50 kDa

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Eng et al PLoS One. 2017 May 17;12(5):e0177834. doi: 10.1371/journal.pone.0177834. eCollection 2017. Fig 5. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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

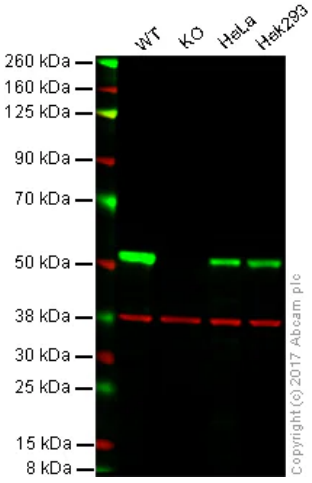
Beta III Tubulin Western blot staining using rabbit Anti-beta III Tubulin antibody

**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.

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

Predicted band size: 50 kDa

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

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

**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

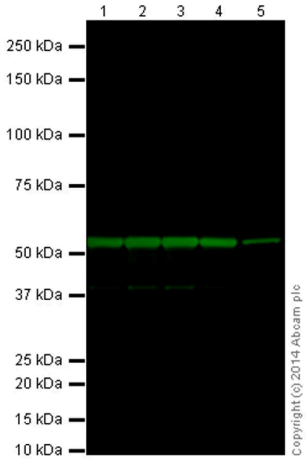
Lane 2:
Beta III Tubulin knockout HAP1 whole cell lysate at 20 µg

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] - Neuronal Marker (ab52623)

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

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

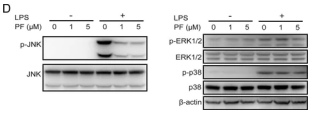



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## Western blot - Anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker (ab52623)

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

beta III Tubulin western blot using anti-beta III Tubulin antibody [EP1569Y] - Neuronal Marker ab52623. Publication image and figure legend from Zhang, H., Qi, Y., et al., 2017, Sci Rep, PubMed 28165507.

ab52623 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab52623 please see the product overview.

PF inhibited IL-6 production of BMDCs and impaired Th17 cell differentiation via suppressing IKK/NF-κB and MAPK signaling pathway in vitro.Bone marrow cells from naïve mice were stimulated by GM-CSF and IL-4 to induce DC differentiation in the presence or absence of the indicated concentrations of PF for 5 days, and followed by the stimulation of LPS. (A) The above DCs were co-cultured with naïve CD4+ T cells for Th17 cell differentiation. The percentage of Th17 cells were analyzed by flow cytometry. (B) The protein expression of IKK/NF-κB signaling pathway was analyzed by immunoblot assay. Tubulin served as control. (C) The nucleus translocation of NF-κB-p65 was monitored by fluorescence microscopy (400×). Nuclei were stained in blue; the NF-κB p65 was stained in red. (D) Protein expression of MAPK signaling pathway was detected by immunoblot assay. β-actin was detected as control. Data are representative of three independent experiments. Values were expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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