

## Product datasheet

# Anti-beta III Tubulin antibody [EPR1568Y] ab68193

KO VALIDATED Recombinant RabMAB

★★★★☆ 1 Abreviews 17 References 10 Images

### Overview

<b>Product name</b>	Anti-beta III Tubulin antibody [EPR1568Y]
<b>Description</b>	Rabbit monoclonal [EPR1568Y] to beta III Tubulin
<b>Host species</b>	Rabbit
<b>Specificity</b>	according to BLAST analysis, it is possible that the antibody will cross-react with TBB6 (Q9BUF5), TBB2B (Q9BVA1), TBB2A (Q13885), TBB5 (P07437), TBB4A (P04350), TBB4B (P68371). This cross-reactivity has not been confirmed experimentally.
<b>Tested applications</b>	<b>Suitable for:</b> ICC/IF, Flow Cyt (Intra), WB, IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide within Human beta III Tubulin aa 1-100 (N terminal). The exact sequence is proprietary.
<b>Positive control</b>	WB: SH-SY5Y, HeLa, Human cerebellum, Mouse brain and Rat brain lysate Flow Cyt(intra): HeLa cells ICC/IF: Neuro-2a cells, Hap1-TUBB3 WT/KO IHC-P: Human tonsil, Mouse and rat testis tissue sections
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAB<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAB<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
<b>Purity</b>	Protein A purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR1568Y
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab68193 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
ICC/IF		1/50. For the unpurified format use at 1-5 µg/ml
Flow Cyt (Intra)		1/20. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For the unpurified format use at 1/1000 dilution
WB		1/50000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa). For the unpurified format use at 1/20000 to 1/100,000 dilution
IHC-P		1/8000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. For the unpurified format use at 1/250 to 1/500 dilution

## 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 maintenance.

**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** Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs

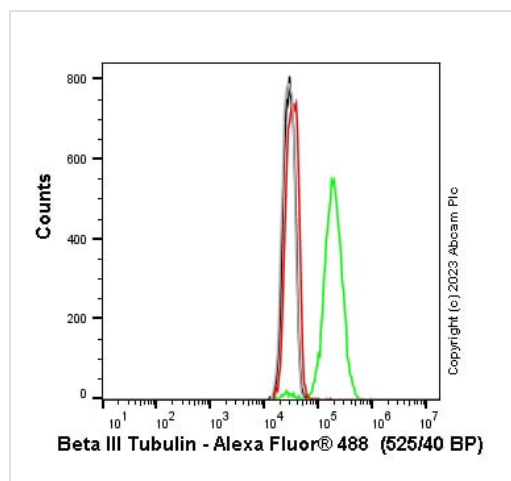
## modifications

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 TTL10 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

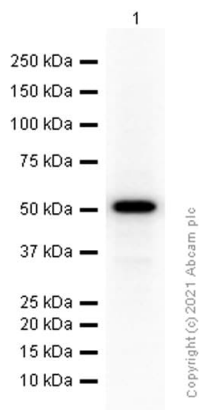


Flow cytometry overlay histogram showing wild-type HeLa (green line) and TUBB3 knockout HeLa stained with ab68193 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab68193) ( $1 \times 10^6$  in 100 $\mu$ l at 0.04  $\mu$ g/ml (1/49250)) 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 HeLa - black line, TUBB3 knockout HeLa - 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.



Western blot - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

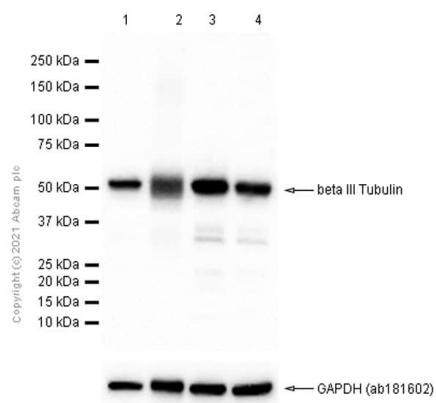
Anti-beta III Tubulin antibody [EPR1568Y] (ab68193) at 1/50000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15  $\mu$ g

**Secondary**

Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

**Predicted band size:** 50 kDa

**Observed band size:** 50 kDa



Western blot - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

**All lanes :** Anti-beta III Tubulin antibody [EPR1568Y] (ab68193) at 1/50000 dilution (Purified)

**Lane 1 :** SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate

**Lane 2 :** Human cerebellum lysate

**Lane 3 :** Mouse brain lysate

**Lane 4 :** Rat brain lysate

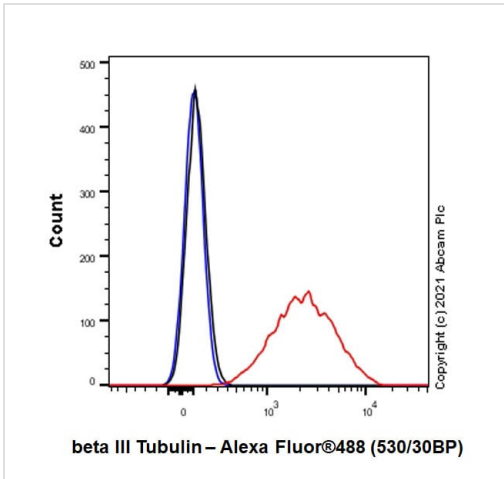
Lysates/proteins at 20  $\mu$ g per lane.

**Secondary**

**All lanes :** Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

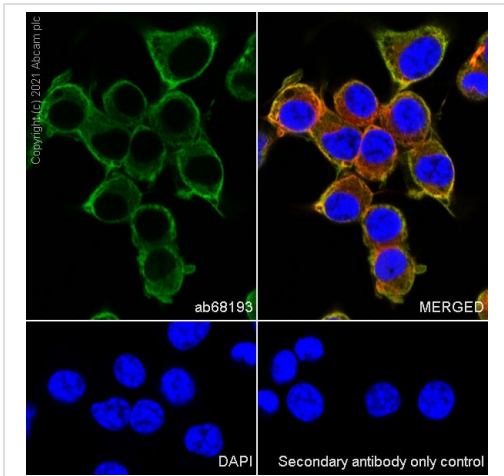
**Predicted band size:** 50 kDa

**Observed band size:** 50 kDa



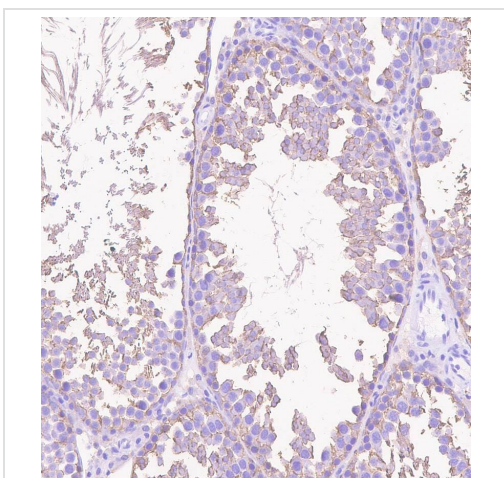
Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling beta III Tubulin with Purified ab68193 at 1:20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



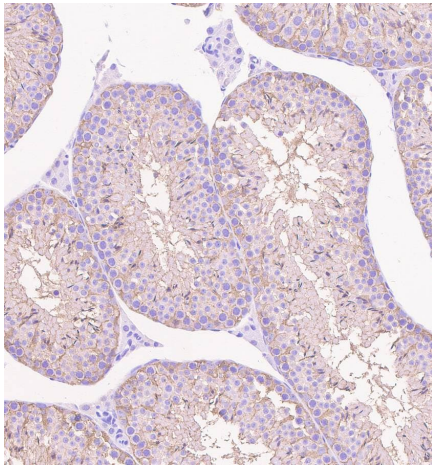
Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

Immunocytochemistry analysis of Neuro-2a(Mouse neuroblastoma neuroblast) cells labeling beta III Tubulin with Purified ab68193 at 1:50 dilution (2.2 µg/ml). Cells were fixed in 100% Methanol and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



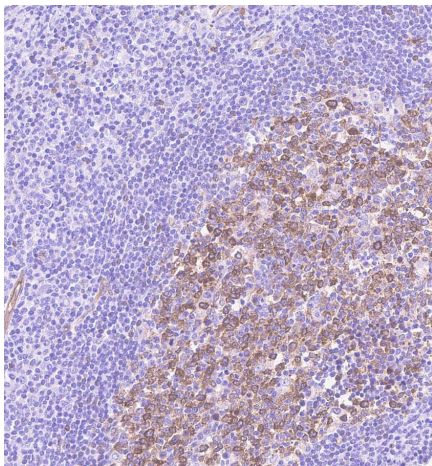
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat testis tissue sections labeling beta III Tubulin with Purified ab68193 at 1:8000 dilution (0.014 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



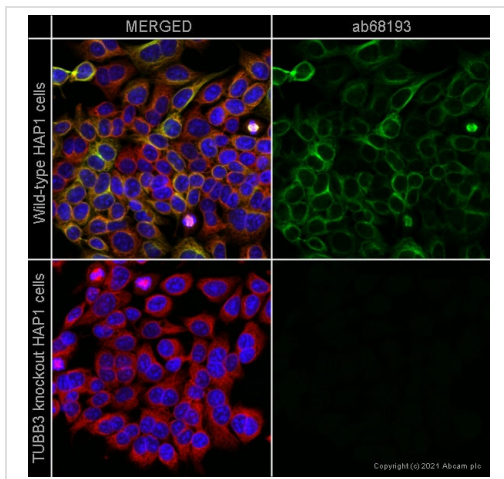
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse testis tissue sections labeling beta III Tubulin with Purified ab68193 at 1:8000 dilution (0.014  $\mu\text{g}/\text{mL}$ ). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling beta III Tubulin with Purified ab68193 at 1:8000 dilution (0.014  $\mu\text{g}/\text{mL}$ ). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.







Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

ab68193 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 ab68193 at 1µg/ml concentration and **ab7291**, a mouse anti-tubulin antibody, at 1µg/ml overnight at +4°C. This is followed by a further incubation at room temperature for 1h with a goat anti-rabbit IgG Alexa Fluor® 488 (**ab150081**) at 2µg/ml (shown in green) and a goat anti-mouse IgG Alexa Fluor® 647 (**ab150119**) at 2µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

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

Why choose a recombinant antibody?

 <p><b>Research with confidence</b> Consistent and reproducible results</p>	 <p><b>Long-term and scalable supply</b> Recombinant technology</p>
 <p><b>Success from the first experiment</b> Confirmed specificity</p>	 <p><b>Ethical standards compliant</b> Animal-free production</p>

Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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