# abcam

### Product datasheet

## Anti-beta III Tubulin antibody [EPR1568Y] ab68193



ייבע RabMAb

★★★★★ 1 Abreviews 17 References 画像数 10

製品の概要

製品名 Anti-beta III Tubulin antibody [EPR1568Y]

製品の詳細 Rabbit monoclonal [EPR1568Y] to beta III Tubulin

由来種 Rabbit

特異性 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.

アプリケーション 適用あり: ICC/IF, Flow Cyt (Intra), WB, IHC-P

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide within Human beta III Tubulin aa 1-100 (N terminal). The exact sequence is

ポジティブ・コントロール 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

特記事項 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**.

製品の特性

製品の状態

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

精製度 Protein A purified

**ポリ/モノ** モノクローナル **ウローン名** EPR1568Y

アイソタイプ IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab68193の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF		1/50. For the unpurified format use at 1-5 μg/ml
Flow Cyt (Intra)		1/20.  ab172730 - Rabbit monoclonal lgG, 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. Fot the unpurified format use at 1/250 to 1/500 dilution

#### ターゲット情報

機能 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.

組織特異性 Expression is primarily restricted to central and peripheral nervous system.

関連疾患 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

配列類似性 Belongs to the tubulin family.

ドメイン The highly acidic C-terminal region may bind cations such as calcium.

翻訳後修飾 Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs

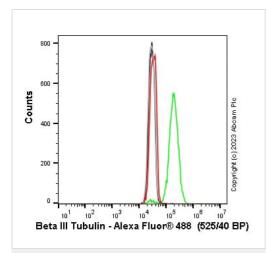
dysmorphism, facial weakness, polyneuropathy.

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.

#### 細胞内局在

Cytoplasm > cytoskeleton.

#### 画像

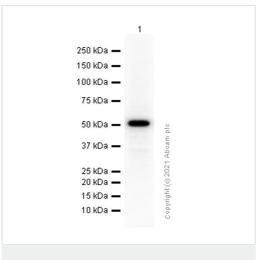


Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

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) (1x  $10^6$  in  $100\mu$ l at  $0.04~\mu$ g/ml (1/49250)) for 30min at  $22^{\circ}$ 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.



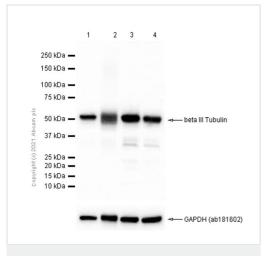
Western blot - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

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

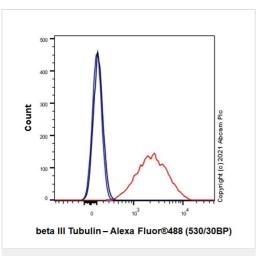
Lane 4: Rat brain lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

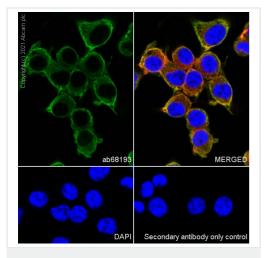
**All lanes :** Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

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



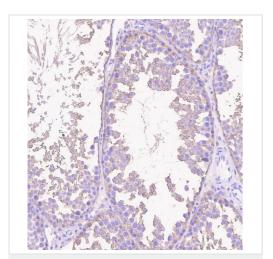
Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling beta Ill 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 lgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



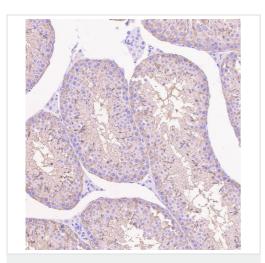
Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody [EPR1568Y] (ab68193)

Immunocytochemistry analysis of Neuro-2a(Mouse neuroblastoma neuroblast) cells labeling beta Ill Tubulin with Purified ab68193 at 1:50 dilution (2.2  $\mu$ g/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  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2  $\mu$ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



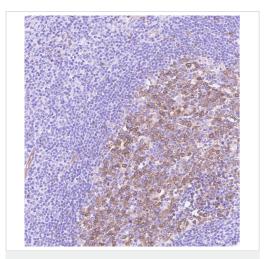
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody [EPR1568Y] (ab68193)

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.



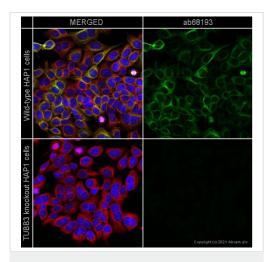
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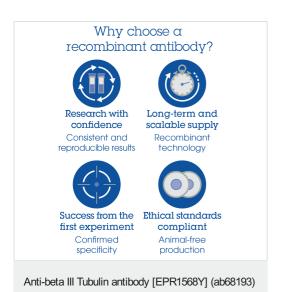
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling beta III Tubulin with Purified ab68193 at 1:8000 dilution (0.014 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval with Citrate buffer (pH 6.0, 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.



Immunocytochemistry/ Immunofluorescence - Antibeta 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 lgG Alexa Fluor® 488 (ab150081) at 2µg/ml (shown in green) and a goat anti-mouse lgG 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).



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