**Product datasheet**

**Anti-beta III Tubulin antibody ab18207**

KO VALIDATED

★★★★★ 53 Abreviews  151 References  15 Images

### Overview

**Product name**  Anti-beta III Tubulin antibody  
**Description**  Rabbit polyclonal to beta III Tubulin  
**Host species**  Rabbit  
**Specificity**  The immunogen used for this product shares 75% homology with TUB (Tubby protein homolog, Uniprot: P50607). In western blot, we observe a specific band at ~55kDa which is not seen in KO cell lines. An additional band below this band of interest is seen at ~50kDa in both the WT and KO cells which could correspond to the protein TUB. Please note that cross-reactivity with this protein has not been confirmed experimentally. TUB is localized notably in high concentrations in the nucleoli of brain neurons with lower protein levels in the cytoplasm. Please, therefore, be aware that ICC experiments may need to be optimised. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above. As an alternative antibody, we would recommend our recombinant rabbit monoclonal antibody ab52623 which has been shown to specific in both WB and ICC using KO cells.

**Tested applications**  Suitable for: IHC-FoFr, Flow Cyt, IHC - Wholemount, IHC-P, IHC-Fr, ICC/IF, WB  
**Species reactivity**  Reacts with: Mouse, Rat, Human, Pig, Rhesus monkey, Common marmoset, Dogfish, Catshark  
**Immunogen**  Synthetic peptide conjugated to KLH derived from within residues 350 to the C-terminus of Human neuron specific beta III Tubulin. Read Abcam’s proprietary immunogen policy (Peptide available as ab18660.)

**Positive control**  Human, Mouse, Rat or Dogfish/Catshark Brain Tissue ICC/IF: SKNSH, Neuro-2A and NGF-differentiated PC12 cells IHC: Hu Cerebellum (FFPE tissue) Flow Cytometry: U-87MG cells, Neuro 2A cells.

### 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 or -80°C. Avoid freeze / thaw cycle.  
**Storage buffer**  Preservative: 0.02% Sodium Azide  
Constituents: 1% BSA, PBS, pH 7.4
**Purity**
Immunogen affinity purified

**Clonality**
Polyclonal

**Isotype**
IgG

## Applications

Our Abpromise guarantee covers the use of **ab18207** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>IHC-FoFr</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration. PubMed: 20568963</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>★★★★★</td>
<td>Use 0.01µg for 10⁶ cells. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody. We recommend <strong>Goat Anti-Rabbit IgG H&amp;L (Alexa Fluor® 488) preadsorbed (ab150081)</strong> secondary antibody</td>
</tr>
<tr>
<td>IHC - Wholemount</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration. PubMed: 25383879</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>Use a concentration of 1 - 5 µg/ml. We recommend <strong>Goat Anti-Rabbit IgG H&amp;L (Alexa Fluor® 488) preadsorbed (ab150081)</strong> secondary antibody</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 50-55 kDa (predicted molecular weight: 50 kDa). Can be blocked with <strong>Human beta III Tubulin peptide (ab18660)</strong></td>
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## 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.

<table>
<thead>
<tr>
<th><strong>Sequence similarities</strong></th>
<th>Belongs to the tubulin family.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Domain</strong></td>
<td>The highly acidic C-terminal region may bind cations such as calcium.</td>
</tr>
<tr>
<td><strong>Post-translational modifications</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Cellular localization</strong></td>
<td>Cytoplasm &gt; cytoskeleton.</td>
</tr>
</tbody>
</table>

| **Images** | |


**Lane 1:** Wild-type HAP1 whole cell lysate (20 µg)

**Lane 2:** Beta III Tubulin knockout HAP1 whole cell lysate (20 µg)

**Lanes 1 - 2:** Merged signal (red and green). Green - ab18207 observed at 55 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab18207 was shown to recognize beta III Tubulin in wild-type HAP1 cells as signal was lost in beta III Tubulin knockout cells. An additional cross-reactive band at 50 kDa was observed in wild-type and knockout cells. Due to the immunogen’s homology with TUB (Tubby protein homolog, Uniprot: P50607), this lower band could correspond to the TUB protein. Please note that cross-reactivity with this protein has not been confirmed experimentally.

Wild-type and beta III Tubulin knockout samples were subjected to SDS-PAGE. Ab18207 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 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 1/20000 dilution for 1 hour at room temperature.
**All lanes**: Anti-beta III Tubulin antibody (ab18207) at 1 µg/ml

**Lane 1**: Human brain tissue lysate - total protein (ab29466)

**Lane 2**: Brain (Mouse) Tissue Lysate

**Lane 3**: Brain (Rat) Tissue Lysate

**Lane 4**: Human brain tissue lysate - total protein (ab29466) with Human beta III Tubulin peptide (ab18660) at 2 µg/ml

**Lane 5**: Brain (Mouse) Tissue Lysate with Human beta III Tubulin peptide (ab18660) at 2 µg/ml

**Lane 6**: Brain (Rat) Tissue Lysate with Human beta III Tubulin peptide (ab18660) at 2 µg/ml

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

**Predicted band size**: 50 kDa

**Observed band size**: 55 kDa

**Exposure time**: 30 seconds
IHC image of ab18207 staining beta III Tubulin in rat cerebellum formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab18207, 1:2000 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
ab18207 staining beta III Tubulin in NGF-differentiated PC12 cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab18207 at 5μg/ml and ab7291 at 1μg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 μg/ml (shown in green) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 2 μg/ml (shown in pseudo color red). Nuclear DNA was labelled 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.
All lanes : Anti-beta III Tubulin antibody (ab18207) at 1 µg/ml

Lane 1 : Brain (Mouse) Tissue Lysate
Lane 2 : Brain (Rat) Tissue Lysate
Lane 3 : Human brain tissue lysate - total protein (ab29466)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 55 kDa

Exposure time: 30 seconds

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 2% Bovine Serum Albumin before being incubated with ab18207 overnight at 4°C. Antibody binding was detected using Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody, and visualised using ECL development solution ab133406.
Immunohistochemistry (Paraffin-embedded sections)
- Anti-beta III Tubulin antibody (ab18207)

IHC image of ab18207 staining beta III Tubulin in Human cerebellum formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab18207, 1μg/ml dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
ab18207 staining beta III Tubulin in Neuro-2a cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab18207 at 1μg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 μg/ml (shown in green) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 2 μg/ml (shown in pseudo color red). Nuclear DNA was labelled 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.

ab18207 staining beta III Tubulin in SKNSH cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab18207 at 1µg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 µg/ml (shown in green) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled 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.
Overlay histogram showing Neuro 2A cells stained with ab18207 (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 (ab18207, 0.01µg/1x10^6) for 30 min at 22°C. The secondary antibody used was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) at 1/4000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (polyclonal) (ab171870, 0.01µg/1x10^6 cells) used under the same conditions. Unlabelled 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.
Overlay histogram showing U-87MG cells stained with ab18207 (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 (ab18207, 0.01μg/1x10⁶) for 30 min at 22℃. The secondary antibody used was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) at 1/4000 dilution for 30 min at 22℃. Isotype control antibody (black line) was rabbit IgG (polyclonal) (ab171870, 0.01μg/1x10⁶ cells) used under the same conditions. Unlabelled 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-87MG cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 20 min used under the same conditions.

ab18207 at 1/2000 staining rat cerebellum tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). The tissue was formaldehyde fixed and a heat mediated antigen retrieval step was performed prior to incubation with the antibody for 16 hours. A biotinylated goat polyclonal antibody was used as the secondary.
Immunocytochemistry/ Immunofluorescence - Anti-beta III Tubulin antibody (ab18207)

ab18207 staining beta III tubulin in PC-12 cells treated with venlafaxine hydrochloride (ab120715), by ICC/IF. Increase in the number and length of neurites (stained with beta III tubulin) correlates with increased concentration of venlafaxine hydrochloride, as described in literature.

The NGF treated cells were incubated at 37°C for 6 hour in media containing different concentrations of ab120715 (venlafaxine hydrochloride) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab18207 (1 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A Goat Anti-Rabbit IgG H&L (DyLight® 488) preadsorbed (ab96899) secondary antibody at 1/250 dilution was used.

Western blot - Anti-beta III Tubulin antibody (ab18207)

All lanes: Anti-beta III Tubulin antibody (ab18207) at 1/1000 dilution

All lanes: Mouse hippocampus tissue lysate

Lysates/proteins at 8 µg per lane.

Secondary

All lanes: Goat anti-rabbit IgG (H&L) at 1/5000 dilution

Predicted band size: 50 kDa

Observed band size: 55 kDa

Exposure time: 10 seconds
ab18207 at 1/2000 staining mouse brain tissue sections by IHC-P. The tissue was formaldehyde fixed and an enzymatic antigen retrieval step was performed prior to incubation with the antibody for 16 hours. A biotinylated goat anti-rabbit IgG was used as the secondary.

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