## abcam

#### Product datasheet

### Anti-beta III Tubulin antibody - Neuronal Marker ab18207



★★★★★ 66 Abreviews 526 References 画像数 17

#### 製品の概要

アプリケーション

ポジティブ・コントロール

免疫原

製品名 Anti-beta III Tubulin antibody - Neuronal Marker

製品の詳細 Rabbit polyclonal to beta III Tubulin - Neuronal Marker

由来種 Rabbit

特異性 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 <u>ab52623</u>

which has been shown to specific in both WB and ICC using KO cells.

種交差性 交差種: Mouse, Rat, Human, Common marmoset, Dogfish, Catshark

交差が予測される動物種: Pig, Rhesus monkey 4

適用あり: ICC/IF, Flow Cyt (Intra), IHC-P, WB

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab18660)

(i childe available as <u>ab rosse</u>)

ovaries, Dogfish/Catshark tissue (snout region). ICC/IF: SK-N-SH cells, Neuro 2A, PC12 and RA induced P19 cells. Primary rat neurons/glia, DN14. WB: WT HAP-1 whole cell lysate. Human,

Flow Cyt (Intra): Neuro 2A, U-87MG cells. IHC-P: Rat cerebellum, mouse brain, adult mouse

mouse and rat brain tissue lysate. Mouse hippocampus tissue lysate.

特記事項
The Life Science industry has been in the grips of a reproducibility crisis for a number of years.
Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies

and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

金ロッ件を

1

製品の特性

製品の状態 Liquid

保存方法 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.

**バッファー** pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

精製度 Immunogen affinity purified

**ポリ/モノ** ポリクローナル

アイソタイプ lgG

#### 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ab18207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	**** (19)	Use a concentration of 1 µg/ml.
Flow Cyt (Intra)		Use 0.01µg for 10 <sup>6</sup> cells.  ab171870 - Rabbit polyclonal lgG, is suitable for use as an isotype control with this antibody.  We recommend Goat Anti-Rabbit lgG H&L (Alexa Fluor <sup>®</sup> 488) preadsorbed (ab150081) secondary antibody.
IHC-P	★★★★☆ (16)	1/2000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	<b>★★★★★ (10)</b>	Use a concentration of 1 µg/ml. Detects a band of approximately 50-55 kDa (predicted molecular weight: 50 kDa).

#### 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 dysmorphism, facial weakness, polyneuropathy.

配列類似性

Dolongo Bolongo

翻訳後修飾

ドメイン

Belongs to the tubulin family.

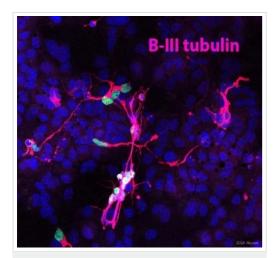
The highly acidic C-terminal region may bind cations such as calcium.

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.

細胞内局在

Cytoplasm > cytoskeleton.

#### 画像

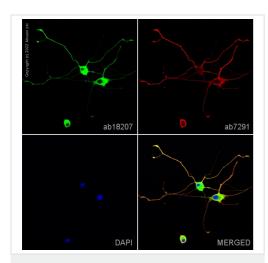


Immunocytochemistry - Anti-beta III Tubulin antibody

- Neuronal Marker (ab18207)

This image is courtesy of an Abreview submitted by Lenin Veeraval

Immunocytochemistry analysis of paraformaldehyde-fixed mouse brain staining with ab18207 at 1/200 dilution. Secondary antibody was Alexa Fluor® 568 Donkey Anti-Rabbit IgG. Samples were incubated with the primary antibody with 2% donkey serum in 0.2% TritonX 100 for 16 hours at 27°C. Blocking was done using 10% donkey serum for 2 hours at 28°C.

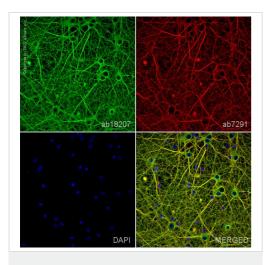


Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody - Neuronal Marker (ab18207)

with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween 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 overnight at 4°C with ab18207 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor<sup>®</sup> 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor<sup>®</sup> 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

ab18207 staining beta III Tubulin in PC12 cells. The cells were fixed

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

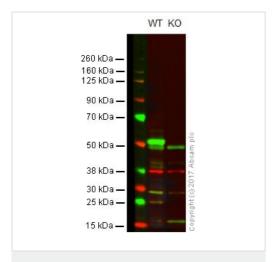


Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody - Neuronal Marker (ab18207)

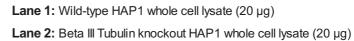
ab18207 staining beta III Tubulin in primary rat neurons/glia, DIV14 (prepared from E18 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDHEP) cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab18207 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled 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,
Perkin Elmer) and a maximum intensity projection of confocal
sections is shown.



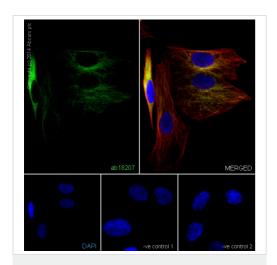
Western blot - Anti-beta III Tubulin antibody -Neuronal Marker (ab18207)



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

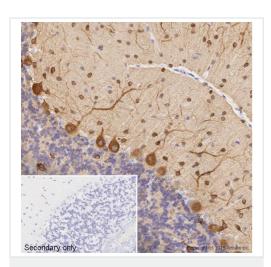
ab18207 was shown to recognize beta III Tubulin in wild-type HAP1 cells as signal was lost in beta III Tubulin knockout cells. An additional cross-reactive band at 50 kDa was observed in wild-type and knockout cells. Due to the immunogen's homology with TUB (Tubby protein homolog, Uniprot: P50607), this 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 lgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed **ab216773** and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature



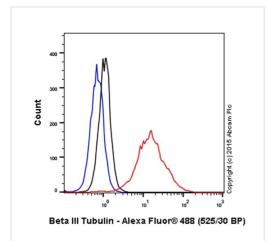
Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody - Neuronal Marker (ab18207)

ab18207 staining beta III Tubulin in SK-N-SH (Human neuroblastoma cell line) 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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)

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.

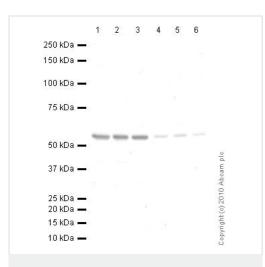


Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)

Overlay histogram showing U-87MG (Human glioblastoma-astrocytoma epithelial cell line) 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<sup>6</sup>) 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<sup>6</sup> 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.



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

All lanes: Anti-beta III Tubulin antibody - Neuronal Marker (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

 $\textbf{Lane 4:} \ \text{Human brain tissue lysate - total protein } (\underline{\textbf{ab29466}}) \ \text{with}$ 

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 lgG - 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

sb 18207 MERGED

DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody - Neuronal Marker (ab18207)

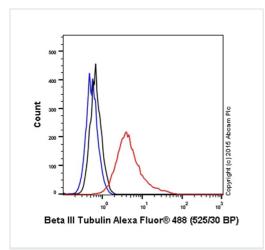
ab18207 staining beta III Tubulin in Neuro-2a cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab18207 at 1µg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further 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.



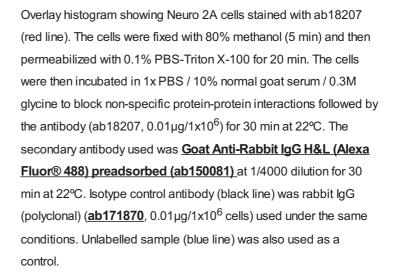
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

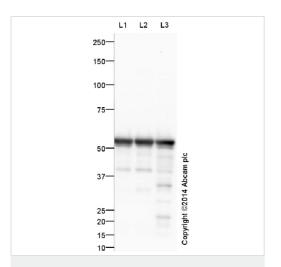
ab18207 at 1/2000 staining mouse brain tissue sections by IHC-P. The tissue was formaldehyde fixed and an enzymatic antigen retrieval step was performed prior to incubation with the antibody for 16 hours. A biotinylated goat anti-rabbit IgG was used as the secondary.



Flow Cytometry (Intracellular) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)



Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-beta III Tubulin antibody -Neuronal Marker (ab18207) **All lanes :** Anti-beta III Tubulin antibody - Neuronal Marker (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) (<u>ab97051</u>) at 1/50000 dilution

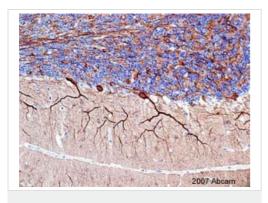
Developed using the ECL technique.

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 (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

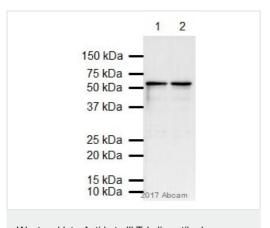
ab18207 at 1/2000 staining rat cerebellum tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). The tissue was formaldehyde fixed and a heat mediated antigen retrieval step was performed prior to incubation with the antibody for 16 hours. A biotinylated goat polyclonal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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

Immunohistochemistical staining (Formaldehyde/PFA-fixed paraffin-embedded sections) for Neuron specific beta III Tubulin antibody - Neuronal Marker (ab18207) on Dogfish/Catshark Tissue sections (head: snout region). Antigen retrieval step: Heat mediated. Blocking step: 1% BSA for 10 mins at RT. Primary Antibody used at 1/2000 incubated for 2 hours at RT. Secondary Antibody: Biotin labelled goat anti rabbit IgG (1/300).



Western blot - Anti-beta III Tubulin antibody -

Neuronal Marker (ab18207)

This image is courtesy of an abreview submitted by Dr Sergi Bayod.

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

All lanes: Mouse hippocampus tissue lysate

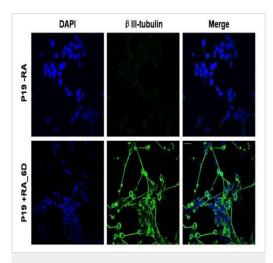
Lysates/proteins at 8 µg per lane.

#### **Secondary**

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

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

Exposure time: 10 seconds



Immunocytochemistry/ Immunofluorescence - Antibeta III Tubulin antibody - Neuronal Marker (ab18207)

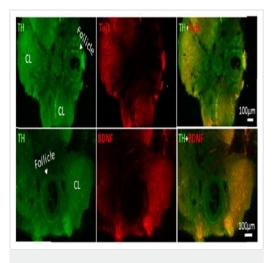
Sheikh, MA et al PLoS One. 2013;8(2):e55826. doi: 10.1371/journal.pone.0055826. Epub 2013 Feb 7 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

# Differential expression of Dnmt1, Dnmt3a, and Dnmt3b during RA induced neuronal differentiation of P19 cells

Mouse P19 cells either left untreated (top panel) or RA treated for initial 2 days and further cultured for 4 days without RA (6 days, bottom panel) were immunostained with neuron specific  $\beta$ -III tubulin antibody and nuclei were stained using DAPI.

In order to confirm the neuronal morphology, the cells were stained for neuron specific beta Ill-tubulin (ab18207). RA induced P19 cells showed immunoreactivity against  $\beta$ Ill-tubulin, indicating a neuronal phenotype. In contrast, undifferentiated P19 cells were  $\beta$ Ill-tubulin negative.

(After Figure 1A of Sheikh et al)



Immunohistochemical analysis of adult mice ovaries undergone Clarity processing staining tyrosine hydroxlase (TH), Beta III Tubulin (Tuj1) with ab18207, and brain derived neurotrpic factor (BDNF) with ab72439. Positive staing of Tuj1 and BDNF is evident in the theca cells and corpus luteum.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta III Tubulin antibody - Neuronal Marker (ab18207)

Courtesy of Feng Y et al. Sci Rep. 2017; 7: 44810. doi: 10.1038/srep44810 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

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

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you

• We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.co.jp/abpromise">https://www.abcam.co.jp/abpromise</a> or contact our technical team.

#### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors