

# Product Datasheet

## beta-III Tubulin Antibody - BSA Free NB100-1612

Unit Size: 0.25 ml

Store at 4C in the dark.

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**NB100-1612**

beta-III Tubulin Antibody - BSA Free

Product Information	
Unit Size	0.25 ml
Concentration	0.3 mg/ml
Storage	Store at 4C in the dark.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgY
Purity	Immunogen affinity purified
Buffer	10mM PBS (0.9% isotonic, w/v, pH 7.2)
Target Molecular Weight	50 kDa

Product Description	
Host	Chicken
Gene ID	10381
Gene Symbol	TUBB3
Species	Human, Mouse, Rat
Marker	Neuron Cell Marker
Immunogen	Chickens were immunized with three synthetic peptide/keyhole limpet hemocyanin (KLH) conjugates. These synthetic peptides corresponded to different regions of beta-III Tubulin, but are shared between the human (NP_AAL28094, NCBI) and rat (AAM28438, NCBI) protein sequences.
Notes	<p>Chicken products cannot be exported to Canada.</p> <p><b>Purification Notes</b> After repeated injections, immune eggs were collected, and the IgY fractions were purified from the yolks. These IgY fractions were then affinity-purified using a peptide column, and the concentrations of the eluates adjusted to 300 ug/ml. Finally, equal volumes of each of the three affinity-purified anti-peptide antibodies were mixed, and the preparation was filter-sterilized.</p> <p><b>Storage Notes</b> Store at 4C in the dark. Under these conditions, the antibodies should have a shelf life of at least 12 months (provided they remain sterile). Do not freeze these antibodies unless you want to store them for longer periods of time. Note, however, that each time an antibody preparation is frozen, about half of its binding activity is lost.</p>

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunohistochemistry Whole-Mount, Knockdown Validated
Recommended Dilutions	Western Blot 1:10000 - 1:20000, Flow Cytometry, Immunohistochemistry 1:2000 - 1:5000, Immunocytochemistry/ Immunofluorescence 1:2000-1:5000, Immunohistochemistry-Paraffin, Immunohistochemistry Whole-Mount, Knockdown Validated

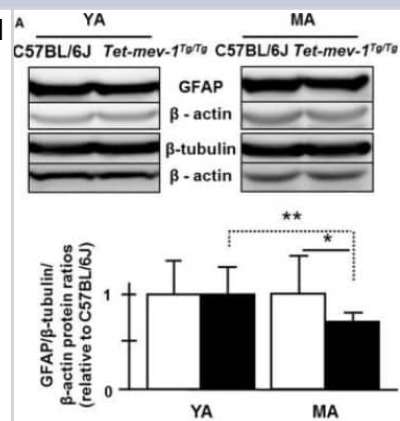


**Application Notes**

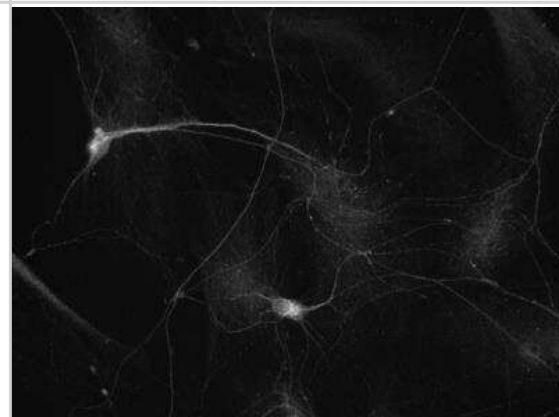
Although not tested, this antibody may be useful for immunohistochemistry on frozen sections. Use in KD reported in scientific literature (PMID:3333046). Each of the three antibodies were analyzed by immunohistochemistry (1:2000). using fluorescein-labeled goat anti-chicken IgY (1:500). as the secondary reagent. Use in Immunohistochemistry-Whole mount reported in scientific literature (PMID 27178445). Use in Immunohistochemistry-Paraffin reported in scientific literature (PMID: 27178445). Use in Flow Cytometry reported in scientific literature (PMID: 31755958).

**Images**

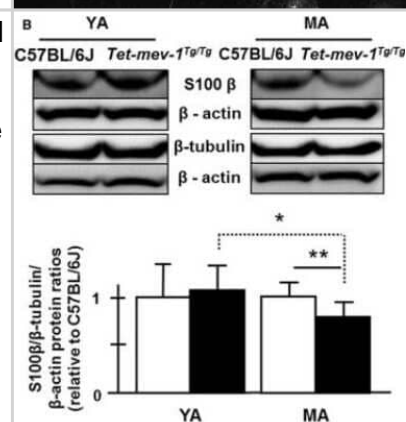
**Western Blot: beta-III Tubulin Antibody [NB100-1612]** - The levels of glial fibrillary acidic protein (GFAP) and S100beta as astrocytic protein marker proteins in the hippocampal area. Western blot images using GFAP, tubulin beta-3, and beta-actin antibodies to total protein lysate in hippocampal area. The statistical results of internal standardized GFAP/beta-tubulin ratio by beta-actin levels relative to young adult wild-type C57BL/6J. White and black bars indicate the wild-type C57BL/6J and Tet-mev-1 mice, respectively. Data are expressed as mean +/- SD; \*P < 0.05; \*\*P < 0.01; n = >12 in each group. Image collected and cropped by CiteAb from the following publication (<https://doi.wiley.com/10.1111/ace.12523>), licensed under a CC-BY license.



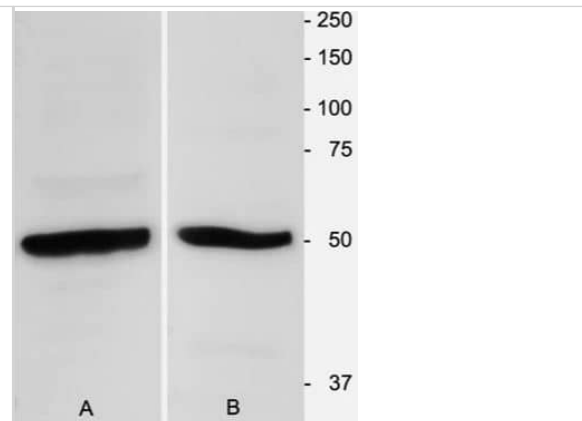
**Immunocytochemistry/Immunofluorescence: beta-III Tubulin Antibody [NB100-1612]** - Beta-III Tubulin in rat primary motor neurons. Ab dilution 1:1000 in PBST (0.1% Triton X-100) + 10% GS O/N at 4C. ICC/IF image submitted by a verified customer review.



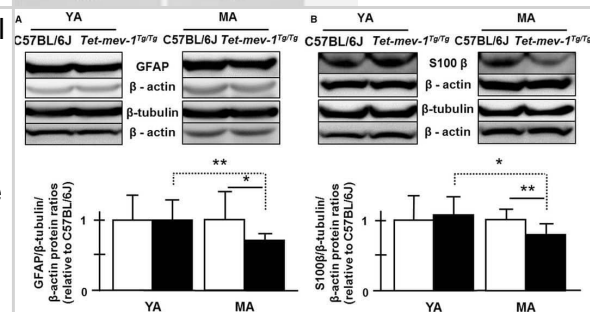
**Western Blot: beta-III Tubulin Antibody [NB100-1612]** - The levels of glial fibrillary acidic protein (GFAP) and S100beta as astrocytic protein marker proteins in the hippocampal area. Western blot images using S100beta, tubulin beta-3, and beta-actin antibodies to total protein lysate in hippocampal area. The statistical results of internal standardized S100beta/beta-tubulin ratio by beta-actin levels relative to young adult wild-type C57BL/6J. White and black bars indicate the wild-type C57BL/6J and Tet-mev-1 mice, respectively. Data are expressed as mean +/- SD; \*P < 0.05; \*\*P < 0.01; n = >12 in each group. Image collected and cropped by CiteAb from the following publication (<https://doi.wiley.com/10.1111/ace.12523>), licensed under a CC-BY license.



beta-III Tubulin Western Blot using homogenates of adult mouse brain (1:3000).



Western Blot: beta-III Tubulin Antibody [NB100-1612] - The levels of glial fibrillary acidic protein (GFAP) & S100 $\beta$  as astrocytic protein marker proteins in the hippocampal area. (A) Western blot images using GFAP, tubulin beta $\beta$ 3, &  $\beta$ -actin antibodies to total protein lysate in hippocampal area. The statistical results of internal standardized GFAP/ $\beta$ -tubulin ratio by  $\beta$ -actin levels relative to young adult wild-type C57BL/6J. White & black bars indicate the wild-type C57BL/6J & Tet $\beta$ mev $\beta$ 1 mice, respectively. Data are expressed as mean  $\pm$  SD; \* $P$  < 0.05; \*\* $P$  < 0.01;  $n$  = >12 in each group. (B) Western blot images using S100 $\beta$ , tubulin beta $\beta$ 3, &  $\beta$ -actin antibodies to total protein lysate in hippocampal area. The statistical results of internal standardized S100 $\beta$ / $\beta$ -tubulin ratio by  $\beta$ -actin levels relative to young adult wild-type C57BL/6J. White & black bars indicate the wild-type C57BL/6J & Tet $\beta$ mev $\beta$ 1 mice, respectively. Data are expressed as mean  $\pm$  SD; \* $P$  < 0.05; \*\* $P$  < 0.01;  $n$  = >12 in each group. (C) Micrographs of immunohistochemical analysis on paraffined hippocampal tissue sections using GFAP & S100 $\beta$  antibody. Brown cells indicate GFAP-stained astrocytes. Scale bar = 100  $\mu$ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/27623715>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Kozhushko N, Beilina A, Cookson MR Generation of gene-corrected isogenic controls from Parkinson's disease patient iPSC lines carrying the pathogenic SNCA p.A53T variant Stem cell research 2023-06-01 [PMID: 37229975]

Details:

1:200 ICC/IF and Flow dilution

De Virgiliis F, Mueller F, Palmisano I et al. The circadian clock time tunes axonal regeneration Cell metabolism 2023-11-03 [PMID: 37951214]

Olguin SL, Patel P, Buchanan CN et al. KHSRP loss increases neuronal growth and synaptic transmission and alters memory consolidation through RNA stabilization Communications biology 2022-07-07 [PMID: 35798971]

E Serger, L Luengo-Gut, JS Chadwick, G Kong, L Zhou, G Crawford, MC Danzi, A Myridakis, A Brandis, AT Bello, F Müller, A Sanchez-Va, F De Virgili, P Liddell, ME Dumas, J Strid, S Mani, D Dodd, S Di Giovanni The gut metabolite indole-3 propionate promotes nerve regeneration and repair Nature, 2022-06-22;0(0):. 2022-06-22 [PMID: 35732737]

Kim MS, Kim DH, Kang HK et Al. Modeling of Hypoxic Brain Injury through 3D Human Neural Organoids Cells 2021-01-25 [PMID: 33504071]

Wrobel, L;Hoffmann, JL;Li, X;Rubinsztein, DC; p37 regulates VCP/p97 shuttling and functions in the nucleus and cytosol Science advances 2024-05-03 [PMID: 38701207] (Western Blot)

Koh J, Liu J, Poon CH et Al. Transplantation of Neural Progenitor Cells Derived from Stem Cells from Apical Papilla Through Small-Molecule Induction in a Rat Model of Sciatic Nerve Injury Tissue Eng Regen Med 2024-06-21 [PMID: 38904732]

Seungyoon B Yu, Haoming Wang, Richard G Sanchez, Natasha M Carlson, Khanh Nguyen, Andrew Zhang, Zachary D Papich, Ahmed A Abushawish, Zachary Whiddon, Weronika Matysik, Jie Zhang, Thomas C Whisenant, Majid Ghassemian, John N Koberstein, Melissa L Stewart, Samuel A Myers, Gulcin Pekkurnaz Neuronal activity-driven O-GlcNAcylation promotes mitochondrial plasticity. Developmental cell 2024-06-04 [PMID: 38843836]

Yi-Zhi Wang, Charlotte C.M. Castillon, Kamil K. Gebis, Elizabeth T. Bartom, Alessandra d'Azzo, Anis Contractor, Jeffrey N. Savas Notch receptor-ligand binding facilitates extracellular vesicle-mediated neuron-to-neuron communication Cell reports 2024-03-15 [PMID: 38241148]

Tomoki Sekimori, Kohji Fukunaga, Hideki Oizumi, Toru Baba, Tomoko Totsune, Atsushi Takeda, Takuya Sasaki, Ichiro Kawahata FABP2 is Involved in Intestinal  $\alpha$ -Synuclein Pathologies. Journal of integrative neuroscience 2024-03-01 [PMID: 38419457]

Charysse Vandendriessche, Arnout Bruggeman, Joyce Foroozandeh, Lien Van Hoecke, Pieter Dujardin, Junhua Xie, Griet Van Imschoot, Elien Van Wonterghem, Jonas Castelein, Cristiano Lucci, Lies De Groef, Roosmarijn E. Vandenbroucke The Spreading and Effects of Human Recombinant  $\alpha$ -Synuclein Preformed Fibrils in the Cerebrospinal Fluid of Mice eNeuro 2024-02-21 [PMID: 38383588]

Watts M, Giadone R, Ordureau A et al. Analyzing ER stress response in ALS patient derived motor neurons identifies druggable neuroprotective targets bioRxiv 2023-11-17 [PMID: 38314348] (ICC/IF, Human)

More publications at <http://www.novusbio.com/NB100-1612>

## Procedures

### Immunohistochemistry Chicken IgY Protocol (NB100-1612)

#### Citrate Buffer Antigen Retrieval Protocol

Background: Formaldehyde fixation (2% or 4%, or as a component of 10% formalin) produces protein cross-links in tissues that tends to interfere with antibody penetration. This seems to be particularly true of paraffin-embedded formaldehyde-fixed tissue. Since chicken IgY antibodies are larger than rabbit or mouse IgG's, "extra steps" may be necessary to compensate for their larger size.

The citrate-based "antigen retrieval" protocol outlined below has been shown to improve chicken IgY antibody penetration into 4% formaldehyde-fixed paraffin-embedded sections, and can increase the degree and intensity of immunoreactivity and immunostaining.

Reagents (NOTE: You can use either the Sodium Citrate or Citric Acid Buffers in step #3, below)

"Sodium Citrate Buffer" (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)

Weigh out 2.94 grams of trisodium citrate (dihydrate). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.00 with 1.0 N HCl. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Citric Acid Buffer" (10mM Citric Acid, 0.05% Tween 20, pH 6.0)

Weigh out 1.92 grams of citric acid (anhydrous). Dissolve in approximately 900 mls of deionized, distilled water. Adjust the pH to 6.0 with 1.0 N NaOH. Add 0.5 ml of Tween-20. Mix. Bring up the volume to 1.0 litres with water. Store this solution at room temperature for 3 months or at 4C for longer periods.

"Phosphate-Buffered Saline" [PBS, 10 mM Sodium phosphate-buffered (pH 7.2) isotonic (0.9%, w/v) saline solution]  
 PBS Tween (0.05% Tween 20 in PBS)  
 Ethanol (80%, 90%, 95%, 100%) diluted with water.

Xylene

Procedure (for use with paraffin-embedded sections):

- 1 Deparaffinize tissue sections in 2 changes of xylene (5 minutes each).
  2. Hydrate in 2 changes of 100% ethanol (3 minutes each), 95% ethanol (1 minute), 90% ethanol (1 minute), 80% ethanol (1 minute). Rinse in distilled water.
  3. Pre-heat steamer or water bath with staining dish containing either Sodium Citrate Buffer or Citrate Buffer. Wait until temperature reaches 95-100 degrees C.
- NOTE: Microwave or pressure cooker can be used as an alternative as a heating source.
4. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes (optimal incubation times will vary).
  5. Remove the staining dish, and allow it to cool to room temperature (for 20 minutes or so).
  6. Rinse sections in PBS Tween twice for 2 minutes each time.

NOTE: The remainder of this protocol is meant to be a suggestion, and can be substituted with your regular immunostaining protocol.

7. Block sections for 30 minutes with Blocking buffer diluted 1:10 with water.
8. Incubate sections with primary antibody at appropriate dilution in antibody dilution buffer overnight at 4 degrees C. Since chicken IgY antibodies are larger than mammalian IgG's, this overnight incubation allows more time for antibody penetration into tissue sections.
9. Rinse sections with PBS Tween 20 twice for 5 minutes each time.
10. Incubate sections with labeled secondary antibody (see NOTE, below) at appropriate dilution (for one hour at room temperature) in a 1:100 dilution of blocking buffer (diluted in PBS).
11. Rinse with PBS Tween 20 for three times for 5 minutes each time.

NOTE: This protocol may use HRP- or fluorescently-labeled secondary antibodies produced in goats or rabbits.







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### **Products Related to NB100-1612**

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BAF010	Goat anti-Chicken IgY Secondary Antibody [Biotin]
NB7276	Goat anti-Chicken IgM Heavy Chain Secondary Antibody
NBP2-22901	Recombinant Human beta-III Tubulin His Protein
1129-ER-050	ErbB2/Her2 [Unconjugated]

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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