

# Anti-Tubulin alpha Picoband™ Antibody (monoclonal, 7B12)

Catalog Number: M03989-3

#### About TUBA1A/TUBA1B/TUBA1C

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. Microtubules of the eukaryotic cytoskeleton perform essential and diverse functions and are composed of a heterodimer of alpha and beta tubulins. The genes encoding these microtubule constituents belong to the tubulin superfamily, which is composed of six distinct families. Genes from the alpha, beta and gamma tubulin families are found in all eukaryotes. The alpha and beta tubulins represent the major components of microtubules, while gamma tubulin plays a critical role in the nucleation of microtubule assembly. There are multiple alpha and beta tubulin genes, which are highly conserved among species. This gene encodes alpha tubulin and is highly similar to the mouse and rat Tuba1 genes. Northern blot studies have shown that the gene expression is predominantly found in morphologically differentiated neurologic cells. This gene is one of three alpha-tubulin genes in a cluster on chromosome 12q. Mutations in this gene cause lissencephaly type 3 (LIS3) - a neurological condition characterized by microcephaly, intellectual disability, and early-onset epilepsy caused by defective neuronal migration. Alternative splicing results in multiple transcript variants encoding distinct isoforms.

#### Overview

Product Name	Anti-Tubulin alpha Picoband™ Antibody (monoclonal, 7B12)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Tubulin alpha Picoband™ Antibody (monoclonal, 7B12) catalog # M03989-3. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 7B12
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Mouse
Uniprot ID	Q71U36

#### **Technical Details**

Immunogen	E.coli-derived human Tubulin alpha recombinant protein (Position: N18-A403).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b





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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.  If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.  Some PubMed article(s) citing the expression level of this target are as follows:  Boster Bio's internal QC testing used:  Western blot, 0.1-0.25ug/ml, Human, Mouse, Rat  Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse, Rat  Immunocytochemistry/Immunofluorescence, 5ug/ml, Human  Flow Cytometry, 1-3ug/1x10 <sup>6</sup> cells, Human



## Anti-Tubulin alpha Picoband™ Antibody (monoclonal, 7B12) (M03989-3) Images

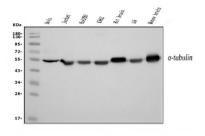


Figure 1. Western blot analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human HELA whole cell lysates,

Lane 2: human Jurkat whole cell lysates,

Lane 3: human HEK293 whole cell lysates,

Lane 4: human K562 whole cell lysates,

Lane 5: rat brain tissue lysates,

Lane 6: rat C6 whole cell lysates,

Lane 7: mouse brain tissue lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Tubulin alpha antigen affinity purified monoclonal antibody (Catalog # M03989-3) at 0.25 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Tubulin alpha at approximately 56KD. The expected band size for Tubulin alpha is at 56KD.

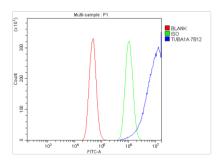


Figure 10. Flow Cytometry analysis of A431 cells using anti-Tubulin alpha antibody (M03989-3).

Overlay histogram showing A431 cells stained with M03989-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Tubulin alpha Antibody (M03989-3,  $1ug/1x10^6$  cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG ( $1ug/1x10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

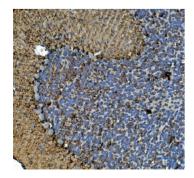


Figure 2. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.





Figure 3. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.



Figure 4. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.



Figure 5. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

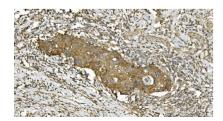


Figure 6. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.





Figure 7. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse lgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.



Figure 8. IHC analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in paraffin-embedded section of human pancreatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

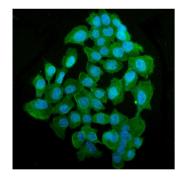


Figure 9. IF analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

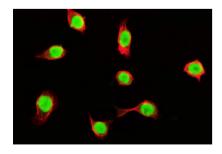


Figure 11. IF analysis of Tubulin alpha using anti-Tubulin alpha antibody (M03989-3).

Tubulin alpha was detected in immunocytochemical section of CACO-2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL mouse anti-Tubulin alpha Antibody (M03989-3) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



- 1. PubMed ID: 10.1186/1477-5956-8-49, A proteomic view of Caenorhabditis elegans caused by short-term hypoxic stress
- 2. PubMed ID: 10.2147/IJN.S291286, Protein Nanoparticle-Related Osmotic Pressure Modifies Nonselective Permeability of the Blood-Brain Barrier by Increasing Membrane Fluidity
- 3. PubMed ID: -, Yu-Yuan Chen, Yin-Peng Bai, Bin Li, Xiao-Bo Zhao, Cheng-Jie Yang, Ying-Qian Liu, Jian-Mei Gao, Jun Guo, Chun Li, Jing-Wen Peng, Zhong-Min Zhao, Zhi-Jun Zhang, Chuan-Rui Xu, Design and Synthesis of Novel 20(S)-alpha-aminophosphonate Derivatives of Camptothecin as Potent Antitumor Agents, Bioorganic Chemistry, 2021, 105065, ISSN 0045-2068, https://doi.org/10.1016/j.bioorg.2021.105065.

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