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Monoclonal Antibody to alpha Tubulin / TUBA1A / TUBA3 - Purified

Alternate names:	Alpha-tubulin 3, Tubulin B-alpha-1, Tubulin alpha-1A chain, Tubulin alpha-3 chain
Catalog No.:	AM39110PU-N
Quantity:	0.1 mg
Concentration:	1.0 mg/ml (lot-specific)
Background:	Tubulin is one of the main components of the cytoskeleton. It is a heterodimeric structure consisting of interlocking alpha and beta chains. Like other cytoskeleton proteins, tubulin undergoes dynamic changes depending on the cellular context, which are mediated by binding and release of GDP/GTP. Tubulin proteins may also undergo post-translational modifications, and are essential to cellular transport and mitotic processes.
Uniprot ID:	P02552
NCBI:	9031
GeneID:	5777
Host / Isotype:	Mouse / IgG1
Recommended Isotype Controls:	SM10P (for use in human samples), SM20P (for use in rat samples), AM03095PU-N
Clone:	DM1A
Immunogen:	Native chicken brain microtubules
Format:	**State:** Liquid purified IgG1 fraction **Purification:** Protein G chromatography **Buffer System:** 0.1 M Tris-Glycine (pH 7.4), 150 mM NaCl with 0.05% sodium azide
Applications:	**Western Blot**: 0.01 µg/mL of this antibody detected α-tubulin in 10 µg of HeLa cell lysate. **Immunocytochemistry**: A 1:500 dilution from a representative lot detected α-tubulin in NIH/3T3, A431, and HeLa cells. **Immunohistochemistry** on FFPE sections: A 1:10,000 dilution from a representative lot detected α-tubulin in normal human brain tissue, human brain cancer tissue, normal rat hippocampus tissue and normal rat cerebellum tissue. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	The antibody reacts with Human and Mouse and Rat alpha-Tubulin. Predicted to react with Chicken based on 100% sequence homology.
Storage:	Store undiluted at 2-8°C. Shelf life: One year from despatch.

General Readings:

1. Saugstad JA, Yang S, Pohl J, Hall RA, Conn PJ. Interaction between metabotropic glutamate receptor 7 and alpha tubulin. J Neurochem. 2002 Mar;80(6):980-8. PubMed PMID: 11953448.
2. Erickson HP, O'Brien ET. Microtubule dynamic instability and GTP hydrolysis. Annu Rev Biophys Biomol Struct. 1992;21:145-66. PubMed PMID: 1525467.

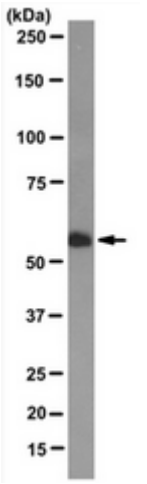
Protocols:

Western Blotting

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA or milk with 0.05% Tween®-20 surfactant for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of the primary antibody, diluted in freshly prepared 5% BSA or milk for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Visualize with enhanced chemiluminescence (ECL) method of choice.

Pictures:

Western Blotting:
HeLa cell lysate was probed with Cat.No. AM39110PU-N, Anti-α-Tubulin, clone DM1A (0.01 µg/mL). Proteins were visualized using a Goat Anti-Mouse IgG (H&L) secondary antibody conjugated to HRP and a chemiluminescence detection system. The arrow indicates □-Tubulin (~60 kDa). Uniprot describes a molecular weight at ~46 kDa, but can be higher due to post-translational modification.



Immunohistochemistry:
Formalin Fixed Paraffin Embedded (FFPE) normal human brain tissue (Fig. 1), human brain cancer tissue (Fig. 2), normal rat hippocampus tissue (Fig. 3) and normal rat cerebellum tissue (Fig. 4) was probed using heat-induced epitope retrieval (HIER). Immunostaining was performed using a 1:10,000 dilution of Cat. No. AM39110PU-N, Anti-α-Tubulin. Reactivity was detected using an Anti-Mouse secondary antibody and HRP-DAB. Positive cytoplasmic staining was observed in neurons and glial cells of normal human brain and human brain cancer tissue. Positive cytoplasmic staining was also observed in neurons of normal rat hippocampus tissue, and in Purkinje and glial cells of normal rat cerebellum tissue. The observed staining in rat brain tissues illustrates high background and may require a 1:10,000 to a 1:20,000 dilution.

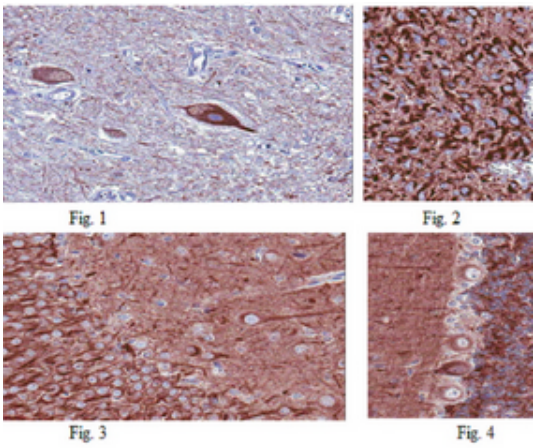


Fig. 1

Fig. 2

Fig. 3

Fig. 4

Confocal fluorescent analysis of NIH/3T3, A431, HeLa cells using Cat. No. AM39110PU-N, Anti-α-Tubulin and a Donkey Anti-Mouse IgG secondary antibody conjugated to Cy3 (Red). Actin filaments have been labeled with Alexa Fluor® 488 dye - Phalloidin (Green). Nucleus is stained with DAPI (Blue). This antibody positively stains the cytoskeleton.