abcam

Product datasheet

# Anti-Tubulin antibody [DM1A +DM1B] - Loading Control ab44928

7 Abreviews 48 References 5 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-Tubulin antibody [DM1A +DM1B] - Loading Control |
| **Description** | Mouse monoclonal [DM1A +DM1B] to Tubulin - Loading Control |
| **Host species** | Mouse |
| **Tested applications** | **Suitable for:** ICC, IHC-P, WB, Flow Cyt |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human, Drosophila melanogaster<br>**Predicted to work with:** Chicken, Guinea pig, Cow, Pig, Gerbil |
| **Immunogen** | Tissue, cells or virus corresponding to Tubulin. Native chick brain microtubules. |
| **Epitope** | aa 426-450 |
| **Positive control** | In Western Blot, this antibody gave a positive signal in the following whole cell lysates: HeLa; NIH3T3; PC12. Ls174T, MAD109 cells. Skin or lung. ICC: Drosophila S2 Schneider cells. |
| **General notes** | 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.<br>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<br>Please note that this antibody is an oligoclonal antibody. It is a cocktail of monoclonal antibodies that have been carefully selected. Oligoclonal antibodies have not only the specificity and batch-to-batch consistency of a monoclonal antibody, but also have the advantage of the sensitivity of a polyclonal antibody due to their ability to recognize multiple epitopes on an antigen. |

## Properties

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| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C or -80°C. Avoid freeze / thaw cycle. |
| **Storage buffer** | pH: 7.40<br>Preservative: 0.1% Sodium azide |

| | |
|---|---|
| | Constituent: PBS |
| **Purity** | Protein G purified |
| **Clonality** | Monoclonal |
| **Clone number** | DM1A +DM1B |
| **Isotype** | IgG1 |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab44928 in the following tested applications.

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

| Application | Abreviews | Notes |
|---|---|---|
| **ICC** | | Use a concentration of 0.2 µg/ml. |
| **IHC-P** | | Use a concentration of 2 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. |
| **WB** | ★★★★★ **(5)** | Use a concentration of 5 µg/ml. Detects a band of approximately 53 kDa (predicted molecular weight: 50 kDa). |
| **Flow Cyt** | | Use 1µg for $10^6$ cells.<br>**ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. |

## Target

| | |
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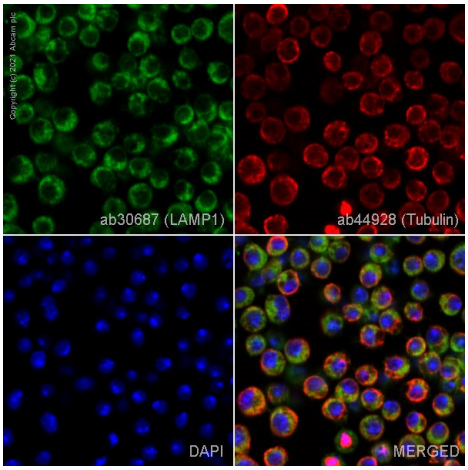
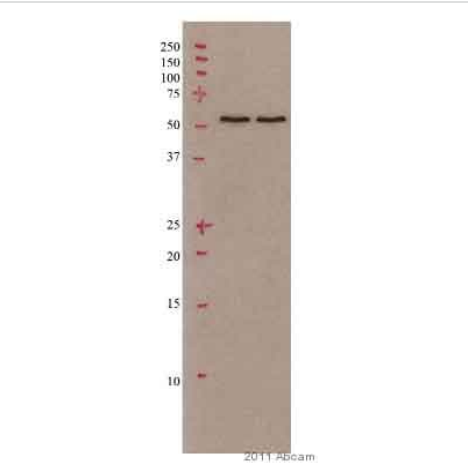
| **Function** | Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. |
| **Sequence similarities** | Belongs to the tubulin family. |
| **Post-translational modifications** | Undergoes a tyrosination/detyrosination cycle, the cyclic removal and re-addition of a C-terminal tyrosine residue by the enzymes tubulin tyrosine carboxypeptidase (TTCP) and tubulin tyrosine ligase (TTL), respectively.<br>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.<br>Acetylation of alpha-tubulins at Lys-40 stabilizes microtubules and affects affinity and processivity of microtubule motors. This modification has a role in multiple cellular functions, ranging from cell motility, cell cycle progression or cell differentiation to intracellular trafficking and signaling. |
| **Cellular localization** | Cytoplasm > cytoskeleton. |

## Images




Immunocytochemistry - Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928)

**ab30687** staining LAMP1 and ab44928 staining tubulin in Drosophila S2 Schneider cells. The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 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 with **ab30687** at 0.2µg/ml concentration and ab44928 at 0.2µg/ml concentration overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).




Western blot - Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928)

Image courtesy of Qifeng Lin by Abreview.

**All lanes :** Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928) at 1/5000 dilution

**All lanes :** Whole cell lysate prepared from human colon cells

Lysates/proteins at 20 µg per lane.

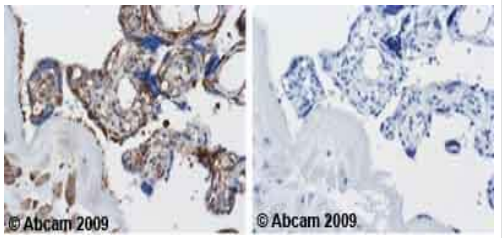
**Secondary**

**All lanes :** HRP sheep anti-mouse polyclonal at 1/10000 dilution

Developed using the ECL technique.

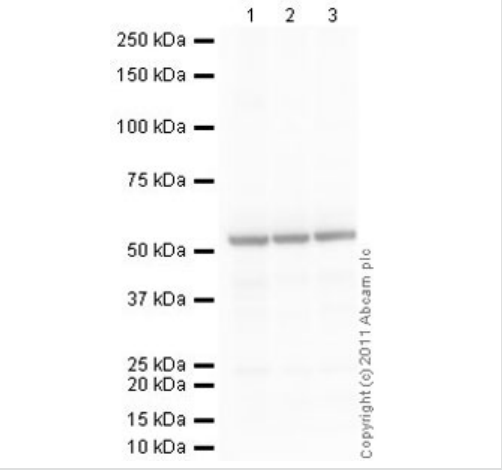
**Predicted band size:** 50 kDa
**Observed band size:** 53 kDa

**Exposure time:** 1 minute




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928)

Ab44928 staining human normal placenta. Staining is localized to the cytoplamsm
Left panel: with primary antibody at 2 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffers EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.




Western blot - Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928)

**All lanes :** Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928) at 5 µg/ml

**Lane 1 :** HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
**Lane 2 :** NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
**Lane 3 :** PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

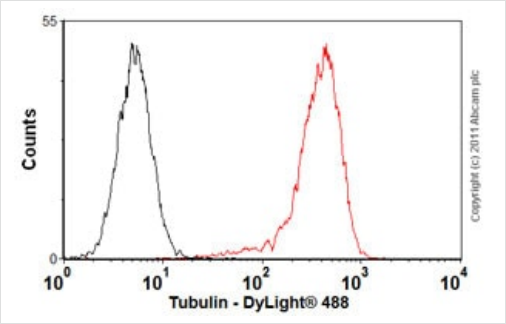
**Secondary**
**All lanes :** Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 50 kDa
**Observed band size:** 53 kDa

**Exposure time:** 2 minutes




Flow Cytometry - Anti-Tubulin antibody [DM1A +DM1B] - Loading Control (ab44928)

Overlay histogram showing HeLa cells stained with ab44928 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween 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. The cells were then incubated with the antibody (ab44928, 1µg/1x$10^6$ cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x$10^6$ cells ) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



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