abcam

Product datasheet

# Anti-alpha Tubulin antibody [DM1A] - Loading Control ab7291

91 Abreviews 1075 References 13 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-alpha Tubulin antibody [DM1A] - Loading Control |
| **Description** | Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control |
| **Host species** | Mouse |
| **Tested applications** | **Suitable for:** ICC/IF, IHC-P, WB, Flow Cyt (Intra) |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human<br>**Predicted to work with:** Chicken, Guinea pig, Hamster, Cow, Dog, Pig, Xenopus laevis, Gerbil, African green monkey |
| **Immunogen** | Full length native protein (purified) corresponding to Chicken alpha Tubulin. |
| **Positive control** | WB: HeLa, HEK293, HepG2, Caco2, NIH3T3, PC12 cell lysates. Flow Cyt (Intra): methanol fixed/Tween permeabilised HeLa cells. ICC/IF: Caco-2, NIH3T3, and SV40LT-SMC cells. IHC-P: Human colon and rat colon tissues. |
| **General notes** | This antibody clone [DM1A] is manufactured by Abcam.<br>Excellent as a protein loading control antibody. DM1A causes the 10 nm filaments to collapse into large lateral aggregates collecting in the cell periphery or tight juxtanuclear caps. It does not block microtubule assembly. It does not inhibit polymerisation or depolymerisation of platelet tubulin in vitro. It blocks (by 70-80%) the ability of tubulin dimers (with GppNHp bound) to promote a stable inhibition of adenylyl cyclase. See references for further information on the above.<br>If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact **orders@abcam.com** or you can find further information **here**.<br>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 |

## Properties

| | |
|---|---|
| **Form** | Liquid |

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|---|---|
| **Storage instructions** | 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. |
| **Storage buffer** | pH: 7.40<br>Preservative: 0.02% Sodium azide<br>Constituents: PBS, 6.97% L-Arginine |
| **Purity** | Protein G purified |
| **Purification notes** | Affinity-purified using protein G |
| **Primary antibody notes** | Excellent as a protein loading control antibody. |
| **Clonality** | Monoclonal |
| **Clone number** | DM1A |
| **Isotype** | IgG1 |
| **Light chain type** | kappa |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab7291 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/IF** | ★★★★★ (25) | Use a concentration of 0.5 - 1 µg/ml. |
| **IHC-P** | ★★★★★ (2) | Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| **WB** | ★★★★★ (60) | 1/5000 - 1/10000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).<br>We recommend diluting ab7291 to 1:10000 and incubating overnight at 4°C. Works under both reducing and non-reducing conditions. We recommend using 3% BSA as the blocking agent, blocking with milk may cause a reduction in signal intensity. |
| **Flow Cyt (Intra)** | | Use 1µg for $10^6$ cells.<br>**ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. |

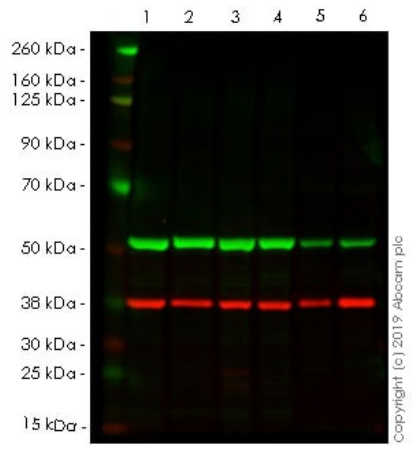
## Target

| | |
|---|---|
| **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** | 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.
Acetylation of alpha chains 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




Western blot - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

**All lanes :** Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution

**Lane 1 :** HeLa cell lysate
**Lane 2 :** PC12 cell lysate
**Lane 3 :** SV40LT-SMC cell lysate
**Lane 4 :** NIH/3T3 cell lysate
**Lane 5 :** Rat liver tissue lysate
**Lane 6 :** Rat heart tissue lysate

Lysates/proteins at 20 µg per lane.

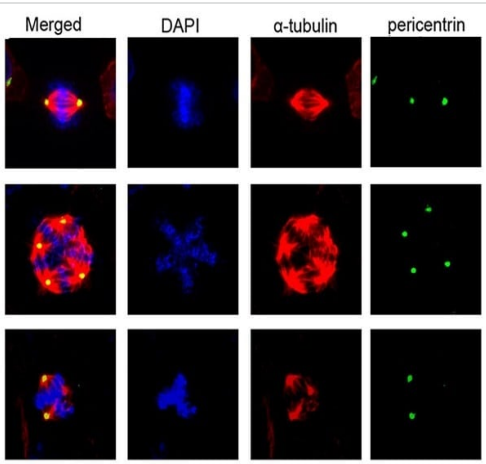
**Secondary**
**All lanes :** Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) at 1/20000 dilution

Performed under reducing conditions.

**Predicted band size:** 50 kDa

Merged signal (red and green). Green - ab7291 observed at 52 kDa. Red - loading control, **ab181602**, observed at 38 kDa.

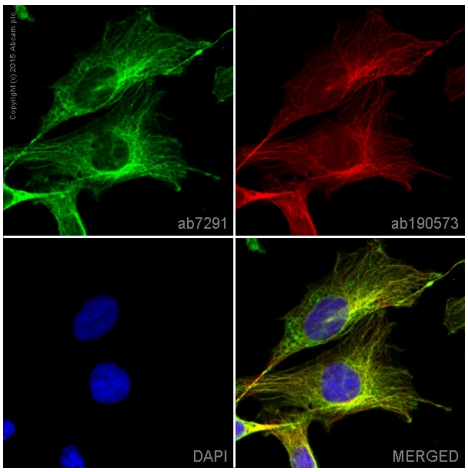
All samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. ab7291 and **ab181602** (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 1/1,000 and 1/20,000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.




Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)



ab7291 staining alpha tubulin in human breast cancer cell line by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed 4% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100 in PBS and blocked with 2% bovine serum albumin in sodium phosphate buffer. Cells were co-stained with anti-pericentrin using **ab4448** at 1:500 dilution and ab7291 at 1:500 dilution. Alexa Fluor® 633 goat anti mouse and Alexa Fluor® 488 goat anti-rabbit (1:500 dilution) was used as secondary antibodies. DAPI was used as a nuclei counterstain. Representative images of mitotic cells with bipolar or multipolar spindles.

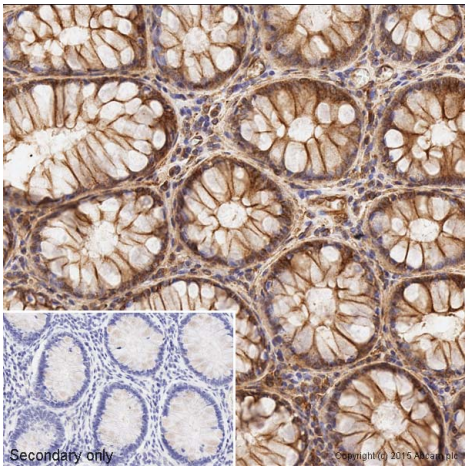



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

ab7291 staining alpha Tubulin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes 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 ab7291 at a working concentration of 0.5µg/ml and **ab190573**, Rabbit monoclonal [EP1332Y] to alpha Tubulin (Alexa Fluor® 647, shown in red) at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-mouse Alexa Fluor® 488 (**ab150117**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

This product also gave a positive signal in 100% methanol (5 min) fixed SV40 cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

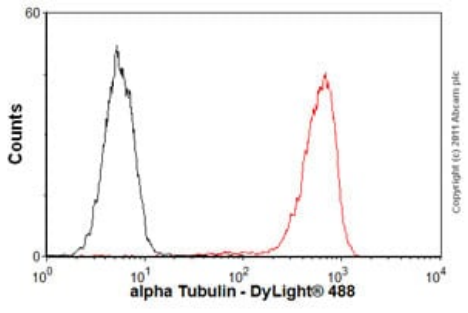



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

IHC image of ab7291 staining alpha Tubulin in human colon 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 ab7291, 5ug/ml working concentration, 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).

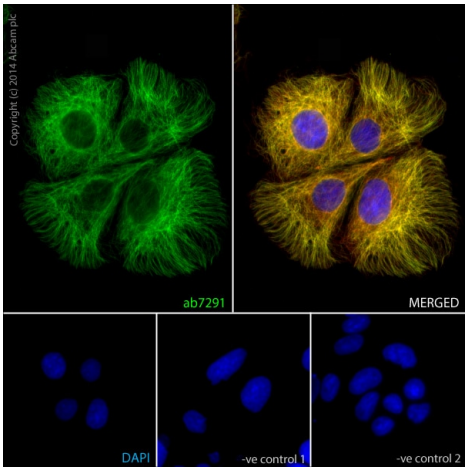
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre




Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

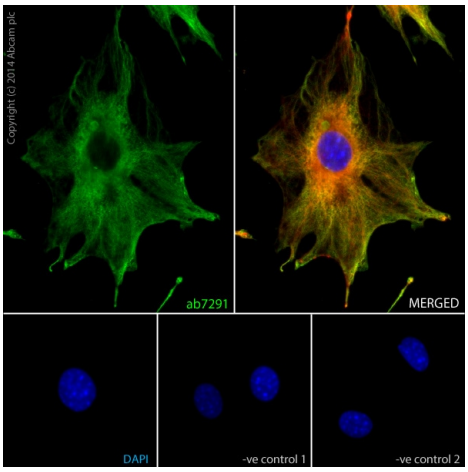
Overlay histogram showing HeLa cells stained with ab7291 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab7291, 1µg/1x$10^6$ cells) for 30 min at 22°C. The secondary antibody used was an anti-mouse DyLight® 488 (**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.




Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

ab7291 staining alpha-Tubulin in Caco-2 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 ab7291 at 1µg/ml and **ab6046** at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-mouse Alexa Fluor® 488 (**ab150117**) at 2 µg/ml (shown in green) and anti-rabbit Alexa Fluor® 594 (**ab150088**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Negative controls:
1 – Rabbit primary antibody 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.




Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

ab7291 staining alpha Tubulin in NIH3T3 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 ab7291 at 1µl/ml and **ab6046** at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-mouse Alexa Fluor® 488 (**ab150117**) at 2 µg/ml (shown in green) and anti-rabbit Alexa Fluor® 594 (**ab150088**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Negative controls:
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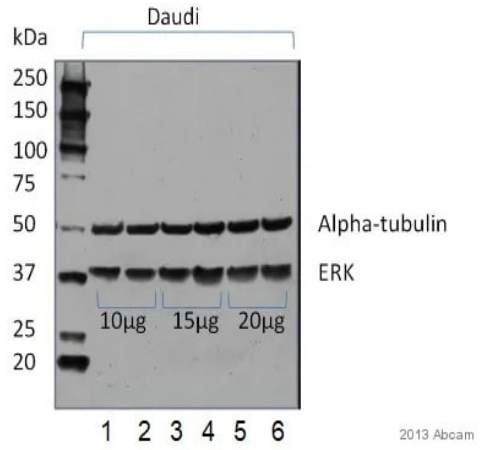
2 – Mouse primary antibody and anti-rabbit secondary antibody.
Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.




Western blot - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

This image is courtesy of an AbReview submitted by Elena Kashuba.

**All lanes :** Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/2500 dilution

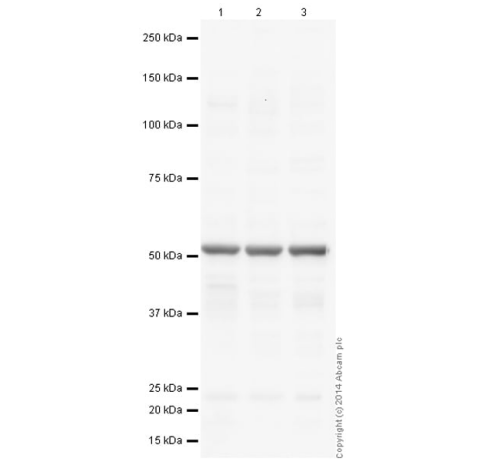
**Lanes 1-2 :** Daudi (Human Burkitt's lymphoma cell line) at 10 µg
**Lanes 3-4 :** Daudi (Human Burkitt's lymphoma cell line) at 15 µg
**Lanes 5-6 :** Daudi (Human Burkitt's lymphoma cell line) at 20 µg

**Secondary**
**All lanes :** HRP conjugated monoclonal Goat Anti-Mouse IgG at 1/1000 dilution

Performed under reducing conditions.

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

**Exposure time:** 1 minute




Western blot - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

**All lanes :** Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1 µg/ml

**Lane 1 :** HeLa (Human epithelial cell line from cervix adenocarcinoma cell line) whole cell lysate
**Lane 2 :** NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
**Lane 3 :** PC12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/50000 dilution

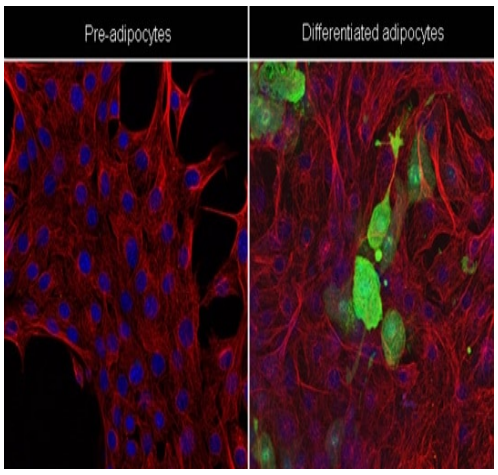
Developed using the ECL technique.

Performed under reducing conditions.

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

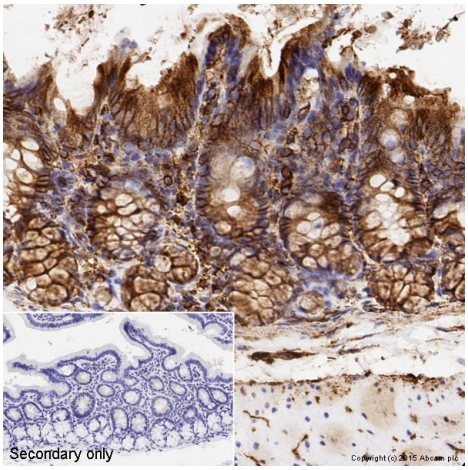
**Exposure time:** 150 seconds

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 ab7291 overnight at 4°C. Antibody binding was detected using an **anti-mouse HRP** (**ab97040**), and visualised using ECL development solution **ab133406**




Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

FABP4 (green) was detected using FABP4 primary antibody (**ab92501**; diluted 1/1000). Alpha tubulin (red) was detected using the mouse monoclonal (ab7291) antibody. Cells were imaged by confocal microscopy, using z-stack for adipocyte-like cells.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

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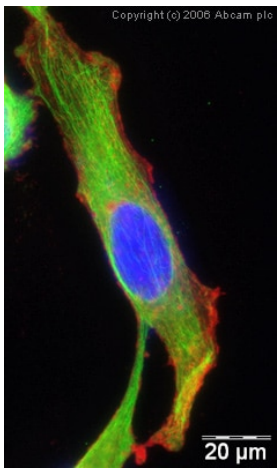
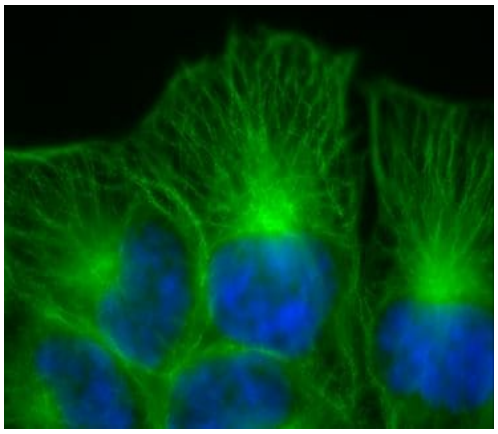



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

ICC/IF image of ab7291 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab7291, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291)

Immunofluorescent imaging of human cells (U2OS) with ab7291 reveals a delicate network of alpha-tubulin (green) located exclusively in the cytoplasm. The nucleus is stained blue.

IF was performed with a standard paraformaldehyde technique (fixed in PBS buffered PFH 4% for 5 minutes, permeabilised with 0.5% triton-PBS for 5 minutes, blocked with 5% milk / 0.2% tween for one hour. Primary antibody used at 1/200 in 5% milk / 0.2% TWEEN for one hour, secondary antibody Alexa 488 for 30 minutes. All blocking and incubation steps carried out at 37 degrees.



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