abcam

ab56676

Anti-Tubulin antibody [4D1] - Loading Control

Key facts

Isotype	IgG2b
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Full Length Protein corresponding to Human TUBA1B. Database link P68363
Clone number	4D1
Purity	Tissue culture supernatant
Light chain type	kappa
Purification notes	Purified from TCS.

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information TUBA1B

Function	Tubulin is the major constituent of microtubules, protein filaments consisting of alpha- and beta-tubulin heterodimers (PubMed:38305685, PubMed:34996871, PubMed:38609661). Microtubules grow by the addition of GTP-tubulin dimers to the microtubule end, where a stabilizing cap forms (PubMed:38305685, PubMed:34996871, PubMed:38609661). Below the cap, tubulin dimers are in GDP-bound state, owing to GTPase activity of alpha-tubulin (PubMed:34996871, PubMed:38609661).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

This product was changed from ascites to tissue culture supernatant on 15 May 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Tubulin sometimes called DM1B is a globular protein essential for the formation of microtubules. It has a molecular mass of approximately 55 kDa. Tubulin is expressed in most eukaryotic cells and exists as two closely related subunits alpha-tubulin and beta-tubulin. These subunits polymerize to form linear microtubule structures. Anti-tubulin antibodies such as anti-tubulin Alexa 34 can detect these microtubule markers in cells allowing researchers to visualize cytoskeletal organization.
Biological function summary	This core component of microtubule assembly is important for maintaining cell shape enabling intracellular transport and segregating chromosomes during cell division. As part of a complex tubulin interacts with various microtubule-associated proteins (MAPs) that regulate its dynamic assembly and disassembly. This regulation is important for processes like axonal transport in neurons and the movement of cilia and flagella in other cell types.
Pathways	Tubulin plays a significant role in the mitotic spindle assembly part of the cell cycle. This process is vital for the accurate segregation of chromosomes to daughter cells. Tubulin interacts with the kinesin and dynein motor proteins within this pathway which are essential for intracellular transport and mitosis. Another key pathway involving tubulin is the intracellular trafficking facilitated by motor proteins which is necessary for maintaining cell homeostasis and function.
Associated diseases and disorders	Aberrant regulation or mutations in tubulin can lead to conditions such as cancer and neurodegenerative diseases. In cancer altered tubulin dynamics contribute to uncontrolled cell proliferation. Neurodegenerative disorders such as Alzheimer's disease show involvement with tubulin through abnormal microtubule stability often linked to the MAP tau protein. Anti-tubulin antibodies including LAMP1 can help in research related to these conditions by offering methods to study tubulin distribution and function in diseased cells.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

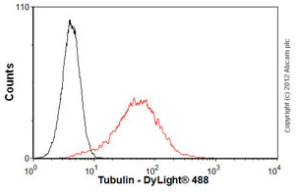
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In the unlikely event of one of our products not working as expected, you are covered by our product promise.

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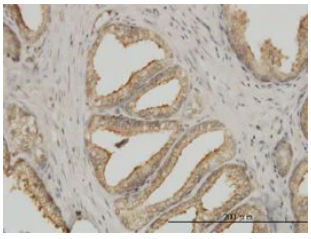
8 product images



Flow Cytometry - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Flow Cytometry staining using mouse Anti-Tubulin antibody

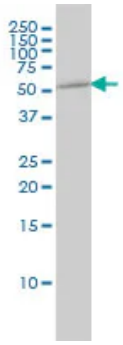
Overlay histogram showing HeLa cells stained with ab56676 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab56676, 2µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.
This image was generated using the ascites version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Tubulin antibody

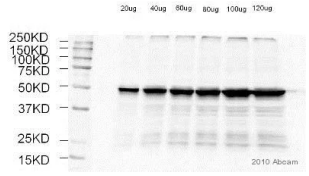
Tubulin antibody (ab56676) used in immunohistochemistry at 3ug/ml on formalin fixed and paraffin embedded human prostate.
This image was generated using the ascites version of the product.



Western blot - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Western blot staining using mouse Anti-Tubulin antibody

Tubulin antibody (ab56676) at 1ug/lane + A-431 cell lysate at 25ug/lane.
This image was generated using the ascites version of the product.



This image is courtesy of a customer review submitted by Xun Ai

Western blot - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Western blot staining using mouse Anti-Tubulin antibody

Blocking Step : 5% BSA for 2 hours at 23°C
This image was generated using the ascites version of the product.

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 1s

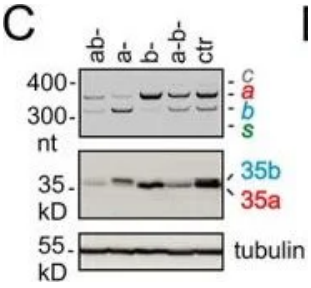


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Western blot - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Western blot staining using mouse Anti-Tubulin antibody

Western Blotting using Anti-Tubulin antibody [4D1] - Loading Control, ab56676. Publication image from Kralovicova, J. et al., 2015, Nucleic Acids Res, 25779042. Legend direct from paper.

Genome-wide identification of U2AF(35)-dependent exons. (A) Alternative splicing of U2AF1 and location of SSOs. Exons are shown as boxes, introns as horizontal lines, SSOs as black rectangles across 3′ss and spliced products (a, b, c, s) as dotted lines. (B) Nucleotide (upper panel) and amino acid (lower panel) sequences of alternatively spliced U2AF1 exons. Amino acids are numbered at the bottom. (C) HinfI digested RT-PCR products showing the relative abundance of U2AF1 isoforms in depleted samples (upper panel) and immunoblot with antibodies against U2AF35 and tubulin (lower panels). ab-, depletion of both isoforms using siRNA U2AF35ab (30 nM); a-, depletion of U2AF35a (60 nM); b-, depletion of U2AF35b (60 nM); a-b-, depletion of U2AF35 using equimolar mixtures of isoform-specific siRNAs; siRNAs were as described (14,23). Ctr, a scrambled control. (D) Normalized expression of U2AF1 and U2AF2 genes in depleted cultures and controls. FPKM, fragments per kilobase of exon model per million reads. Error bars in panels D–F are 95% confidence intervals. U2AF1/U2AF2 ratios in ab-, a-, b- and control cultures were 0.09, 0.43, 1.47 and 2.25, respectively. (E) Normalized expression of U2AF1 isoforms. (F) Normalized expression of U2AF2 isoforms. Extra amino acids included in U2AF65 as a result of alternative GC 5′ss usage are in parentheses. (G) A genome browser view of exon Ab- and 3-containing isoforms in depleted cells (ab-) and controls (ctr). Exons are numbered at the top and corresponding isoforms are shown at the bottom. Browser views are in the native gene orientation throughout; the 5′>3′ transcriptional orientation is denoted by the > sign. (H) Significant sharing of genes identified by DEXSeq (exon-level) and Cufflinks (gene-level) as differentially expressed in ab- cultures versus controls.

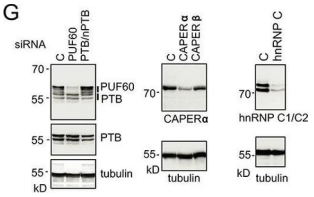


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Western blot - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Western blot staining using mouse Anti-Tubulin antibody

Western Blotting using Anti-Tubulin antibody [4D1] - Loading Control, ab56676. Publication image from Kralovicova, J. et al., 2015, Nucleic Acids Res, 25779042. Legend direct from paper.

Structural and functional characterization of U2AF(35)-dependent 3′ss. (A, D) AGEZ (A) and PPT (D) length of alternative 3′ss (inset) and internal exons affected by U2AF35 depletion. Columns show means, error bars denote SDs. The number of each event is in the legend to Supplementary Figure S8. PPT length was computed for BPs with the highest SVM scores (41). P-values were derived from t-tests; ****P < 0.00005; **P < 0.005. AGEZ and PPT length correlated with the expression change of upregulated (B, E) but not downregulated (C, F) exons; r, Pearson correlation coefficient. (G) Immunoblots prepared from lysates from HEK293 cells depleted of poly(Y)-binding proteins (indicated at the top). Antibodies are shown at the bottom or to the right. (H) Functional antagonism and synergism of Y-binding proteins and U2AF. Exon inclusion levels of each transcript are in Supplementary Figure S11; RT-PCR primers are in Supplementary Table S4. Residual protein levels (RPL) were estimated from immunoblots shown in panel G. The Y-axis is on a log10 scale. Average changes between inclusion levels of upregulated and downregulated exons were compared by the Wilcoxon-Mann-Whitney test (*P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.00005; NS: not significant; -: not tested). (I) Opposite effects of PUF60 depletion and overexpression on a GANAB exon. Immunoblots with the indicated antibodies are to the left and RT-PCR to the right. The GANAB minigene is schematically shown at the top; alternative exon is denoted by a white rectangle. Exogeneous RNA products were amplified by primers PL3 and PL4. Error bars are SDs of duplicate transfections. (J) Positional differences in unpaired probabilities upstream of U2AF(35)-activated and repressed exons. Positive delta PU values signify a higher average single-strandedness of upregulated exons in the optimal BP location and further upstream as compared to downregulated exons whereas negative values reveal their tendency to engage in local base-pairing interactions closer to 3′ss. Stars denote positions with P-values <0.05.

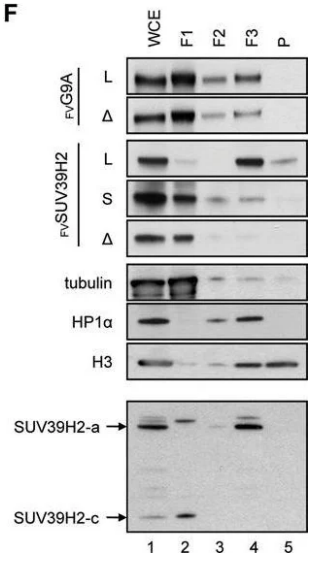


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Western blot - Anti-Tubulin antibody [4D1] - Loading Control (ab56676)

Tubulin Western blot staining using mouse Anti-Tubulin antibody

Western Blotting using Anti-Tubulin antibody [4D1] - Loading Control, ab56676. Publication image from Klinck, R. et al., 2015, Nucleic Acids Res, 25605796. Legend direct from paper.

Protein stability and sub-nuclear localization of SUV39H2 isoforms are regulated by alternative inclusion of exon 3. (A) Expression in HEK 293T cells of ectopic tagged G9A and SUV39H2 protein isoforms. Cells were transduced with the pLVX vector containing Flag-V5 tags and SUV39H2 or G9A cDNA to express exogenous protein isoforms (FVSUV39H2 or FVG9A). Cells transduced with pLVX empty vector were used as controls (Ctl). The ectopic proteins were revealed by western blot using the V5 antibody. (B) and (C) Stability analysis of each G9A and SUV39H2 protein isoform. FVG9A and FVSUV39H2 isoforms were assessed by western blot in HeLa cells treated with cycloheximide (CHX) (B) or MG132 (C) for the indicated times in hours (h). The signal detected with V5 antibody was normalized to histone H3 levels. (D) Localization of FVG9A and FVSUV39H2 isoforms in HeLa cells. Exogenous proteins were revealed with V5 antibody (red), while DNA was counterstained with DAPI (blue). (E) and (F) Analysis of FVG9A and FVSUV39H2 and the endogenous SUV39H2 isoforms after fractionation of HeLa cells. (E) Scheme displaying the cell fractionation procedure. (F) Protein isoforms were detected in whole cell extracts (WCE) and fractions by western blot using the V5 or SUV39H2 antibodies. Endogenous tubulin, HP1α and H3 proteins were analyzed as markers of the cell fractions.

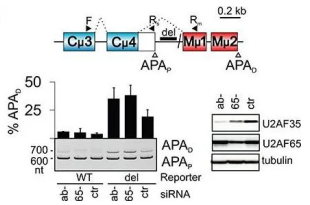


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Tubulin Western blot staining using mouse Anti-Tubulin antibody

Western Blotting using Anti-Tubulin antibody [4D1] - Loading Control, ab56676. Publication image from Kralovicova, J. et al., 2015, Nucleic Acids Res, 25779042. Legend direct from paper.

Characterization of global changes in APA induced by U2AF35 depletion. (A) Distribution of start, internal and terminal exons upregulated and downregulated in cells depleted of U2AF35. P-value was derived from a χ2-test for the 3x2 contingency table. P-value for the first exons versus the pool of internal and terminal exons was <10−7. (B) A high proportion of transcripts with ≥2 differentially expressed exons. (C-E) APA site usage in the indicated APA categories. Number of proximal and distal APA sites altered in depleted cells is shown above arrows that indicate shifts in APA site usage. APAP, APAD, proximal and distal polyadenylation sites. Upregulated and downregulated exons are indicated by red and green rectangles throughout. Each category is schematically shown at the top; yellow rectangles are 3′UTRs, blue boxes are constitutive exons, red and pink boxes are alternative exons. Splicing is denoted by dotted lines. (F) Frequency distribution of APA categories altered in ab- cells. (G) Breakdown of start, internal and terminal exons for Cufflinks-positive genes. APA/5′ss, altered APA or 5′ss of the first intron in ab- cells; ATI, annotated alternative transcription initiation sites altered in ab- cultures. P-value was computed as in panel A. (H) Validation of intronic/alternative 3′ss APA site usage in two plant homology domain-encoding genes shown in panels D and E. PCR primers are in Supplementary Table S4. (I) Control of mouse intronic APA site usage by human U2AF35. Schematics of the mouse IgM minigene with APA sites giving rise to membrane (APAD) and soluble (APAP) immunoglobulins M (upper panel). Del, deletion of the RNA polymerase II (polII) pausing site (100). The lower panel shows RNA products of the wild-type and deletion-containing IgM minigenes transfected into HEK293 cells individually depleted of each U2AF subunit. Final concentration of U2AF65 siRNA was 40 nM. Immunoblot is to the right.

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