abcam

ab52866

Anti-alpha Tubulin antibody [EP1332Y] - Loading Control

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) is a rabbit monoclonal antibody detecting alpha Tubulin in Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF. Suitable for Drosophila melanogaster, Human, Mouse, Pig, Rat. - Biophysical QC for unrivalled batch-batch consistency - Over 240 publications - Trusted since 2007

Lab Essentials | Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP1332Y
Purification technique	Affinity purification Protein A
Specificity	This antibody is expected to recognise most alpha tubulin proteins and not only TUBA4A.
Concentration	0.67 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Pig
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Drosophila melanogaster
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/50000
Notes	-

Species	Rat
Dilution info	1/1000 - 1/50000

Notes	-

Species	Pig
Dilution info	1/1000 - 1/50000
Notes	-

Species	Human
Dilution info	1/1000 - 1/50000
Notes	-

Expected

Species	Drosophila melanogaster
Dilution info	1/1000 - 1/50000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250 - 1/500
Notes	-

Expected

Species	Mouse
Dilution info	-
Notes	-

Species	Rat
Dilution info	-
Notes	-

Species	Pig
Dilution info	-

Notes	-

Species	Drosophila melanogaster
Dilution info	1/250 - 1/500
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20 - 1/50
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	-
Notes	-

Species	Rat
Dilution info	-
Notes	-

Species	Pig
Dilution info	-
Notes	-

Species	Drosophila melanogaster
Dilution info	1/20 - 1/50
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information TUBA4A

Function	Tubulin is the major constituent of microtubules, a cylinder consisting of laterally associated linear protofilaments composed of alpha- and beta-tubulin heterodimers. Microtubules grow by the addition of GTP-tubulin dimers to the microtubule end, where a stabilizing cap forms. Below the cap, tubulin dimers are in GDP-bound state, owing to GTPase activity of alpha-tubulin.

Storage

Shipped at conditions	Conditional Ambient
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

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, WB in drosophila melanogaster, human, mouse, pig, rat samples.

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) has been cited over 240 times in peer reviewed journals and is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (Anti-TUBA4A antibody) (ab52866) has 28 independent reviews from customers.

alpha Tubulin antibodies are often used as loading controls in Western Blot. Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker has been verified in Western Blot samples and detects a band at 50kDa Molecular weight.

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) specifically detects alpha Tubulin (UniProt ID: P68366; Molecular weight: 50kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone EP1332Y - ab216650.

Antibody clone EP1332Y is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 594, PE, Alexa Fluor® 555 (ab185031, ab190573, ab202272, ab208752, ab275113).

Alpha Tubulin (TUBA4A) is a fundamental component of microtubules, which are crucial for maintaining the cytoskeleton and facilitating cell division. Abnormalities in alpha Tubulin are linked to neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, and Huntington's Disease, as well as cancer, highlighting its disease relevance. Post-translational modifications of alpha Tubulin play a significant role in cellular functions and disease mechanisms. Our TUBA4A antibody is a valuable tool for studying these pathways and understanding the molecular basis of these conditions.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

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

Activity summary	Alpha tubulin also known as DM1A is an important component of the structural unit called microtubules. This protein weighs approximately 50 kDa and acts as a building block in the cytoskeleton. Alpha tubulin is expressed in various cell types across many organisms playing a fundamental role in maintaining cell shape and enabling intracellular transport. Researchers often acetylate alpha tubulin to study post-translational modifications and its effects on microtubule dynamics.
Biological function summary	Alpha tubulin combines with beta tubulin to form the tubulin dimer which is the basic unit of microtubule polymerization. These dimers assemble into microtubule filaments integral in numerous cellular processes. As part of the cytoskeleton network alpha tubulin facilitates roles in mitosis and meiosis providing spindle structures necessary for chromosome separation. Its participation in cell signaling pathways is noteworthy as it interacts with motor proteins like kinesins and dyneins to aid cellular activities.
Pathways	Alpha tubulin holds critical roles in microtubule-associated processes within the cytoskeleton and intracellular transport pathways. It interacts with the MAP (microtubule-associated protein) family which modulates microtubule stability and impacts cell division and trafficking. Moreover the microtubule pathway involves proteins like tau which bind to stabilize microtubules and are implicated in cellular dynamics and signaling.
Associated diseases and disorders	Alpha tubulin is relevant in neurodegenerative diseases such as Alzheimer's disease and cancer. Aberrant modifications or mutations in alpha tubulin or associated proteins like tau can disrupt microtubule stability contributing to pathological conditions. In cancer altered expression of alpha tubulin affects cell

division leading to tumor progression and chemoresistance. Researchers study alpha tubulin using techniques such as immunofluorescence with Alexa fluor 555 to understand its role in disease better.

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

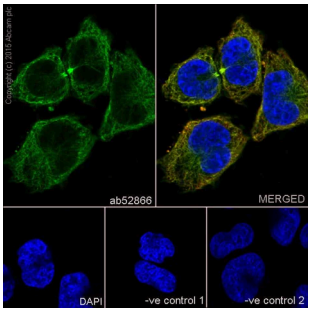
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

Full details and terms and conditions can be found here:
Terms & Conditions.

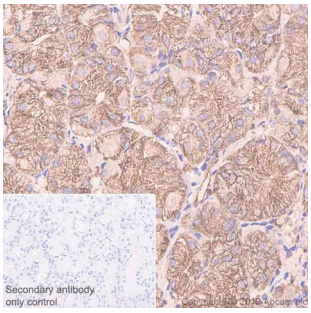
35 product images



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-alpha Tubulin antibody

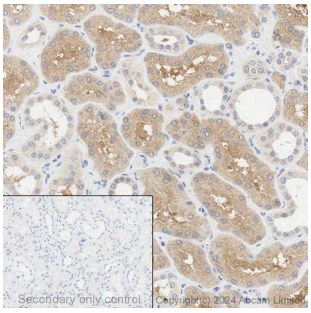
Immunofluorescent analysis of 4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling alpha Tubulin with ab52866 at 1/500 dilution. The cells were permeabilised with 0.1% Triton X-100. Anti-rabbit Alexa Fluor® 488 (ab150077) at 1/400 dilution was used as the secondary antibody (green). The confocal image shows microtubules staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 and anti-mouse AlexaFluor® 594 (ab150120) at 1/500 dilution (red). The negative controls are as follows:
1. ab52866 at 1/500 dilution followed by anti-mouse AlexaFluor® 594 (ab150120) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution followed by anti-rabbit Alexa Fluor® 488 (ab150077) at 1/400 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

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

Immunohistochemical analysis of paraffin-embedded Human stomach tissue labeling alpha Tubulin with ab52866 at followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on human stomach.The section was incubated with ab52866 for 30 mins at room temperature. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human kidney using rabbit Anti-alpha Tubulin antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human kidney staining alpha Tubulin with ab52866 at a concentration of 0.01 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab52866 anti- alpha Tubulin [EP1332Y] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)

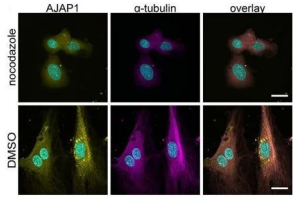


Image from Hotte K. et al Biol Open. 2017 Jun 15;6(6):723-731. doi: 10.1242/bio.022335.

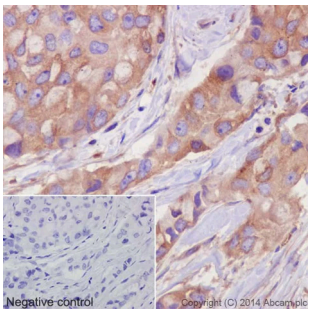
Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-alpha Tubulin antibody

AJAP1 co-localizes with microtubules in HUVECs
The association of AJAP1 with microtubules in HUVECs is lost upon microtubule destruction. Treatment with 12.5 µM nocodazole for 24 h shows destruction of the microtubule network and loss of AJAP1 tubular localization. For a negative control, HUVECs are treated with DMSO for 24 h. Cell nuclei were counterstained with DAPI (cyan). Microscope: Zeiss LSM 780; objective lens: 63×/1.40 oil; scale bar: 25 µm.

Incubated overnight at 4°C with ab52866.

(From Figure 3E of Hotte et al)

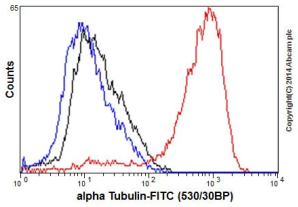


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

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

Immunohistochemistry analysis of paraffin-embedded Human breast cancer labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on cancer cells shown. Secondary antibody ab97051 Goat Anti-Rabbit IgG H&L (HRP) used at a 1/500 dilution. Counter stained with Hematoxylin.
Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (ab97051) at 1/500 dilution.

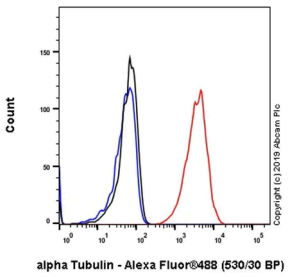
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Flow Cytometry (Intracellular) staining using rabbit Anti-alpha Tubulin antibody

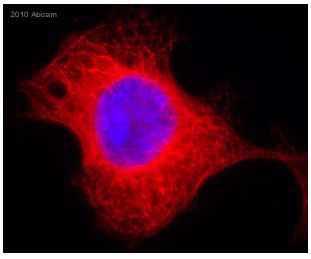
Intracellular Flow Cytometry analysis of 2% paraformaldehyde fixed HepG2 (human liver hepatocellular carcinoma cell line) cells labeling alpha Tubulin with ab52866 at 1/130 dilution (red line). Secondary antibody used is a goat anti rabbit IgG (FITC) at 1/150 dilution. The isotype control is rabbit monoclonal IgG (black line). The unlabeled control is cells without incubation with primary and secondary antibodies (blue line).



Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Flow Cytometry (Intracellular) staining using rabbit Anti-alpha Tubulin antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HepG2 (Human hepatocellular carcinoma epithelial cell)cells labelling alpha Tubulin with ab52866 at 1/2000 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730)isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

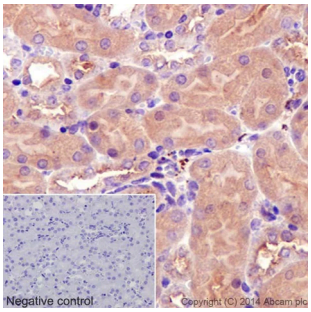


This image is courtesy of an anonymous customer review.

Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-alpha Tubulin antibody

ab52866 staining alpha Tubulin in 293 Human embryonic kidney cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and blocked with 10% serum for 2 hours at 23°C. Samples were incubated with primary antibody (1/200 in 0.5% saponin) for 2 hours at 23°C. An Alexa Fluor®555-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody. Nuclei were counterstained with DAPI.



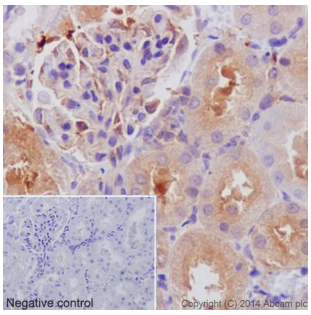
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

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

Immunohistochemistry analysis of paraffin-embedded Mouse kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Mouse kidney tubule shown. Secondary antibody Anti-Rabbit HRP (ab97051) used at a 1/500 dilution. Counter stained with Hematoxylin.

Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

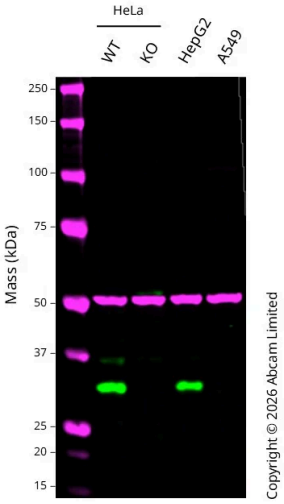


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

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

Immunohistochemistry analysis of paraffin-embedded Rat kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Rat kidney tubule and weak on glomerulus shown. Secondary antibody Anti-Rabbit HRP (ab97051) used at a 1/500 dilution. Counter stained with Hematoxylin.
Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-MTAP antibody [MTAP/1813] ab238024 staining at 1 µg/mL, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 31kDa in Wild-type HeLa cell lysates with no signal observed at this size in MTAP knockout HeLa cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-MTAP antibody [MTAP/1813] (ab238024) at 1 µg/mL

Lane 1:
Wild-type HeLa cell lysates at 20 µg

Lane 2:
MTAP knockout HeLa cell lysates at 20 µg

Lane 3:
HepG2 cell lysates at 20 µg

Lane 4:
A549 cell lysates at 20 µg

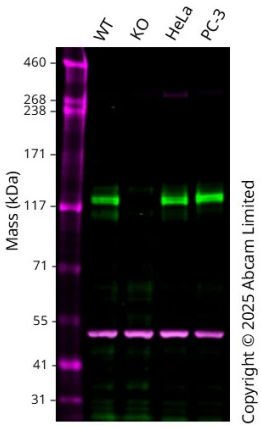
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 31 kDa

Observed band size: 31 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: anti-ACK antibody staining at 1/1000 dilution, shown in green; Rabbit anti alpha Tubulin (ab52866) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 118 kDa in Wild-type HCT 116 cell lysates with no signal observed at this size in TNK2 knockout HCT 116 cell line (ab301274). To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot at 1/1000 dilution

Lane 1:
Wild-type HCT 116 at 20 µg

Lane 2:
Western blot - Human TNK2 knockout HCT116 cell line (ab301274) at 20 µg

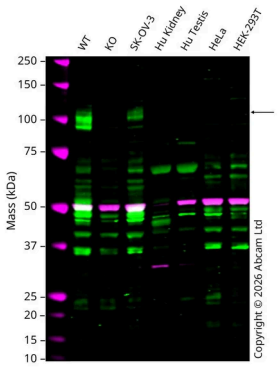
Lane 3:
HeLa at 20 µg

Lane 4:
PC-3

Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-TIM 1 antibody [3D9F5] ab233720 staining at 1/500 dilution, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 100 kDa in Wild-type A549 not boiled cell lysates with no signal observed at this size in HAVCR1 knockout A549 not boiled cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween 20 (TBS-T) before incubation with primary antibodies overnight at 4 C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:

Western blot - Anti-TIM 1 antibody [3D9F5] (ab233720) at 1/500 dilution

Lane 1:
Wild-type A549 not boiled at 20 µg

Lane 2:
HAVCR1 knockout A549 not boiled at 20 µg

Lane 3:
SK-OV-3 not boiled at 20 µg

Lane 4:
Human Kidney not boiled at 20 µg

Lane 5:
Human Testis not boiled at 20 µg

Lane 6:
HeLa not boiled at 20 µg

Lane 7:
HEK-293T not boiled at 20 µg

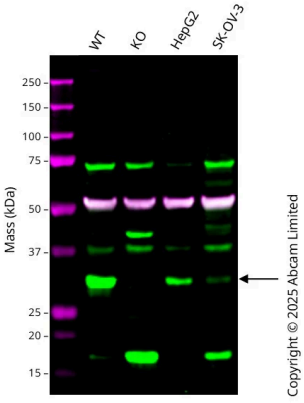
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 100 kDa

Observed band size: 100 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-TIGAR antibody staining at 1/1000 dilution, shown in green; Rabbit anti alpha Tubulin (ab52866) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 30 kDa in Wild-type A549 cell lysates with no signal observed at this size in TIGAR knockout A549 cell line (ab326027). To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Anti-TIGAR antibody at 1/1000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
Western blot - Human TIGAR knockout A549 cell line (ab326027) at 20 µg

Lane 3:
HepG2 at 20 µg

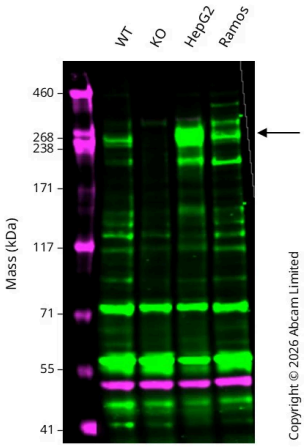
Lane 4:

SK-OV-3 at 20 µg

Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-ABCA1 antibody [AB.H10] ab18180 staining at 1/500 dilution, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 254 kDa in Wild-type HAP1 UNBOILED cell lysates with no signal observed at this size in ABCA1 knockout HAP1 UNBOILED cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-ABCA1 antibody [AB.H10] (ab18180) at 1/500 dilution

Lane 1:
Wild-type HAP1 UNBOILED at 20 µg

Lane 2:
ABCA1 knockout HAP1 UNBOILED at 20 µg

Lane 3:
HepG2 UNBOILED at 20 µg

Lane 4:
Ramos UNBOILED at 20 µg

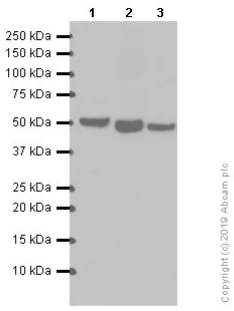
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 254 kDa

Observed band size: 254 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Blocking/Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/10000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) Whole cell lysates at 15 µg

Lane 2:
PC-12 (Rat adrenal gland heochromocytoma) whole cell lysates at 15 µg

Lane 3:
NIH/3T3(Mouse embryonic fibroblast)whole cell lysates at 15 µg

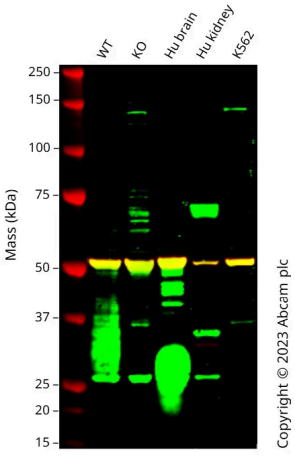
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 50 kDa

Observed band size: 52 kDa

Exposure time: 1s



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Anti-THY1 antibody [7E1B11] (ab181469) staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab181469 was shown to bind specifically to THY1. A band was observed at 25-37 kDa in wild-type U-2 OS cell lysates with no signal observed at this size in THY1 knockout cell line ab262490 (knockout cell lysate ab263925). To generate this image, wild-type and THY1 knockout U-2 OS cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-CD90 / Thy1 antibody [7E1B11] (ab181469) at 1/1000 dilution

Lanes 1, 3, 4 and 5:
Western blot at 20 µg

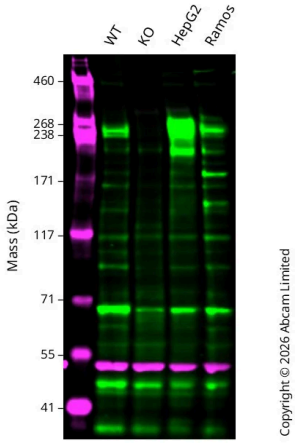
Lane 2:
Western blot - Human THY1 (CD90) knockout U-2 OS cell line (ab262490) at 20 µg

Secondary

All lanes:
Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 17 kDa



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Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-ABCA1 antibody [HJ1] ab66217 staining at 1/1000 dilution, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 254 kDa in Wild-type HAP1 UNBOILED cell lysates with no signal observed at this size in ABCA1 knockout HAP1 UNBOILED cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-ABCA1 antibody [HJ1] (ab66217) at 1/1000 dilution

Lane 1:
Wild-type HAP1 UNBOILED at 20 µg

Lane 2:
ABCA1 knockout HAP1 UNBOILED at 20 µg

Lane 3:
HepG2 UNBOILED at 20 µg

Lane 4:
Ramos UNBOILED at 20 µg

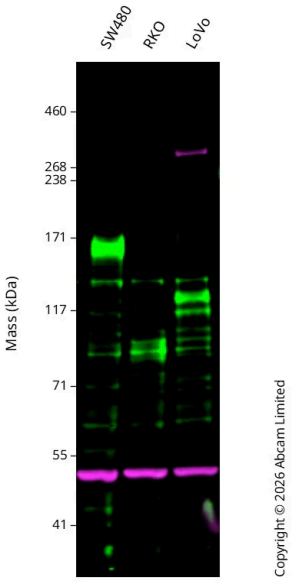
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 254 kDa

Observed band size: 254 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-APC antibody [ALi 12-28] ab58 staining at 1/200 dilution, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 170 kDa. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in LI-COR in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-APC antibody [ALi 12-28] (ab58) at 1/200 dilution

Lane 1:
SW480 cell lysate at 20 µg

Lane 2:
Western blot - Human APC knockout HCT116 cell line (ab277914) at 20 µg

Lane 3:
LoVo cell lysate at 20 µg

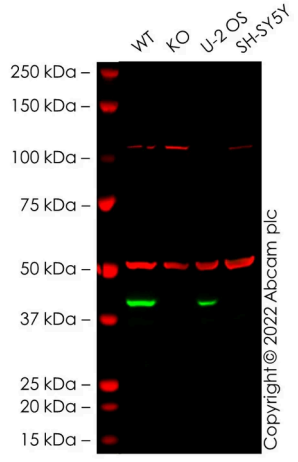
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 90-180 kDa

Observed band size: 170 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

False colour image of Western blot: Anti-MAGEA4 antibody [OTI1F9] staining at 1/4000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab139297 was shown to bind specifically to MAGEA4. A band was observed at 40 kDa in wild-type A431 cell lysates with no signal observed at this size in MAGEA4 knockout cell line ab273857 (knockout cell lysate ab273811). To generate this image, wild-type and MAGEA4 knockout A431 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-MAGEA4 antibody [OTI1F9] (ab139297) at 1/4000 dilution

Lane 1:
Wild-type A431 cell lysate at 20 µg

Lane 3:

U-2 OS cell lysate at 20 µg

Lane 4:
SH-SY5Y cell lysate at 20 µg

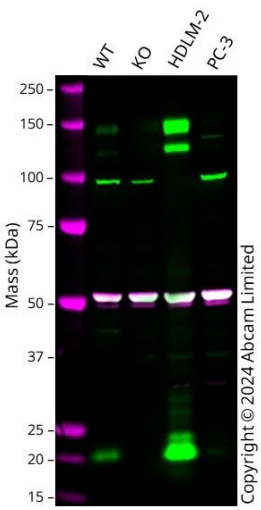
Secondary

All lanes:
Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 35 kDa

Observed band size: 40 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-SEMA4D antibody [30/CD100] (ab307685) staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab307685 was shown to bind specifically to SEMA4D. A band was observed at 90-120 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in SEMA4D knockout cell line. To generate this image, wild-type and SEMA4D knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Semaphorin 4D/CD100 antibody [30/CD100] (ab307685) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
SEMA4D knockout HCT 116 cell lysate at 20 µg

Lane 3:
HDLM-2 UNBOILED cell lysate at 20 µg

Lane 4:
PC-3 UNBOILED cell lysate at 20 µg

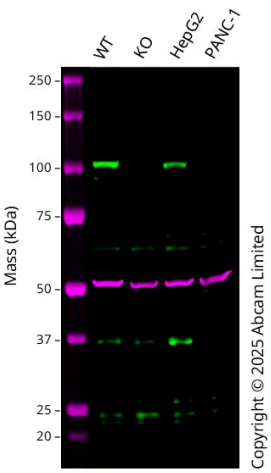
Secondary

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 90-120 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-HEF1/NEDD-9 antibody [2G9] ab18056 staining at 0.1 µg/mL, shown in green; Rabbit anti alpha Tubulin (ab52866) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 100 kDa in Wild-type A549 cell lysates with no signal observed at this size in NEDD9 knockout A549 cell line.

To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-HEF1/NEDD-9 antibody [2G9] (ab18056) at 0.1 µg/mL

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
NEDD9 knockout A549 at 20 µg

Lane 3:
HepG2 at 20 µg

Lane 4:
PANC-1 at 20 µg

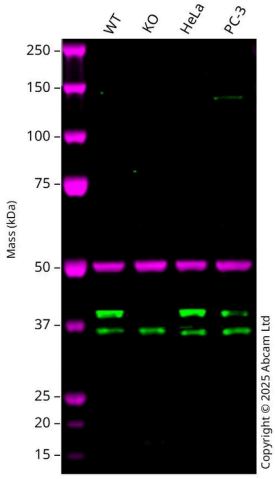
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 93 kDa

Observed band size: 100 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-SLP-2 antibody ab89025 staining at 1 µg/mL, shown in green; Rabbit anti alpha Tubulin (ab52866) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 40 kDa in Wild-type A549 cell lysates with no signal observed at this size in STOML2 knockout A549 cell line.

To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:

Western blot - Anti-SLP-2 antibody (ab89025) at 1 µg/mL

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
STOML2 knockout A549 at 20 µg

Lane 3:
HeLa at 20 µg

Lane 4:
PC-3 at 20 µg

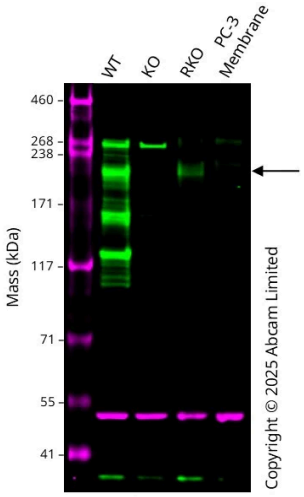
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 40 kDa

Observed band size: 40 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Mouse Monoclonal[2D2] to ARID1B ab57461 staining at 1/1000 dilution, shown in green; Rabbit anti alpha Tubulin (ab52866) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 200 kDa in Wild-type HCT 116 Nuclear cell lysates with no signal observed at this size in ARID1B knockout HCT 116 Nuclear cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-ARID1B antibody [2D2] (ab57461) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 Nuclear at 20 µg

Lane 2:
Western blot - Human ARID1B knockout HCT116 cell line (ab287219) at 20 µg

Lane 3:
RKO at 20 µg

Lane 4:
PC-3 Membrane at 20 µg

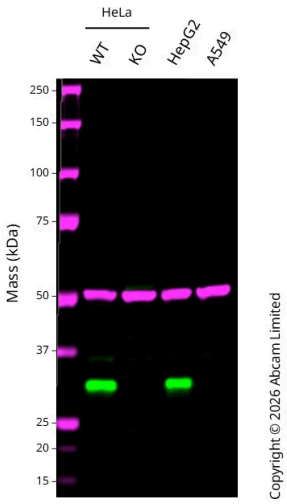
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 244 kDa

Observed band size: 200 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-MTAP antibody [2G4] ab55517 staining at 1 µg/mL, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 31kDa in Wild-type HeLa cell lysates with no signal observed at this size in MTAP knockout HeLa cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-MTAP antibody [2G4] (ab55517) at 1 µg/mL

Lane 1:
Wild-type HeLa cell lysates at 20 µg

Lane 2:
MTAP knockout HeLa cell lysates at 20 µg

Lane 3:
HepG2 cell lysates at 20 µg

Lane 4:
A549 cell lysates at 20 µg

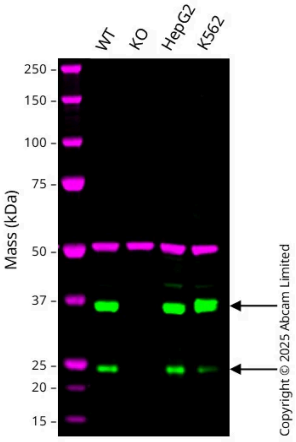
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 31 kDa

Observed band size: 31 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-Crk p38 antibody [22/Crk] ab300630 staining at 1/1000 dilution, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 35, 24 kDa in Wild-type HeLa cell lysates with no signal observed at this size in CRK knockout HeLa cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Crk p38 antibody [22/Crk] (ab300630) at 1/1000 dilution

Lane 1:
Wild-type HeLa whole cell lysate at 20 µg

Lane 2:
Western blot - Human CRK (Crk p38) knockout HeLa cell line (ab265097) at 20 µg

Lane 3:
HepG2 whole cell lysate at 20 µg

Lane 4:
K562 whole cell lysate at 20 µg

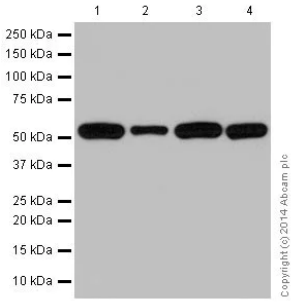
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 35 kDa, 24 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/20000 dilution

Lane 1:
HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate at 20 µg

Lane 3:
Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate at 20 µg

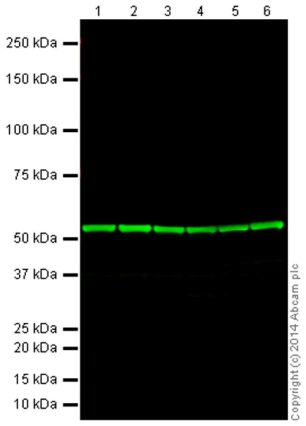
Lane 4:

HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 50 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

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 Licor blocking buffer before being incubated with ab52866 overnight at 4°C. Antibody binding was detected using Anti-Rabbit Alexa Fluor® 790 (ab175781) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.
All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/1000 dilution

Lane 1:
HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 20 µg

Lane 2:
HEK293 (Human embryonic kidney cell line) Whole Cell Lysate at 20 µg

Lane 3:
HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate at 20 µg

Lane 4:
Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate at 20 µg

Lane 5:
NIH/3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate at 20 µg

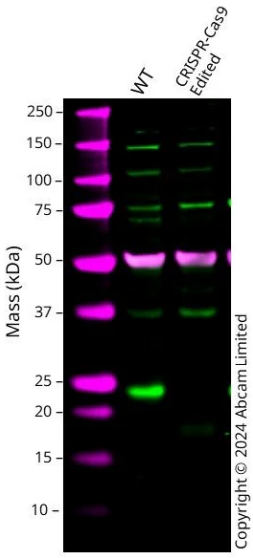
Lane 6:
PC-12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) at 1/10000 dilution

Predicted band size: 50 kDa

Observed band size: 52 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

Western blot: Anti-MGMT antibody [MT3.1] (ab39253) staining at 1/500 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab39253 was shown to bind specifically to MGMT. A band was observed at 22 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in MGMT knockout cell line ab286541. The band observed in the knockout lysate lane below 22 kDa is likely to represent a truncated form of MGMT. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and MGMT knockout HCT 116 cell lysates were analysed.First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-MGMT antibody [MT3.1] (ab39253) at 1/500 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
MGMT knockout HCT 116 cell lysate at 20 µg

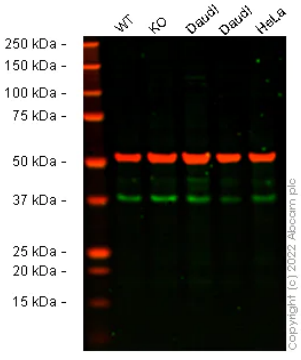
Secondary

Lanes 1 - 2:
Goat anti-Mouse IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 2:
Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 22 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

False colour image of Western blot: Anti-PVRIG/CD112R antibody staining at 1 ug/ml, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in red.In Western blot, ab168153 was found to be non-specific. A band was observed at 38 kDa in wild-type Jurkat cell lysates with no change observed in the PVRIG knockout cell line ab274900 (knockout cell lysate ab274958). To generate this image, wild-type and PVRIG knockout Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.
All lanes:
Western blot - Anti-PVRIG/CD112R antibody (ab168153) at 1 µg/mL

Lane 1:
Wild-type Jurkat cell lysate at 20 µg

Lane 2:
PVRIG knockout Jurkat cell lysate at 20 µg

Lane 3:
Daudi cell lysate at 20 µg

Lane 4:
Daudi cell lysate at 10 µg

Lane 5:
HeLa cell lysate at 20 µg

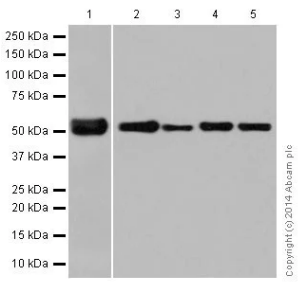
Secondary

Lanes 1 - 5:
Western blot - Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/20000 dilution

Lanes 1 - 5:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 34 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining using rabbit Anti-alpha Tubulin antibody

All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/5000 dilution

Lane 1:
Mouse brain lysates at 10 µg

Lane 2:
C6 (Rat glial tumor cell line) whole cell lysates at 10 µg

Lane 3:
RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysates at 10 µg

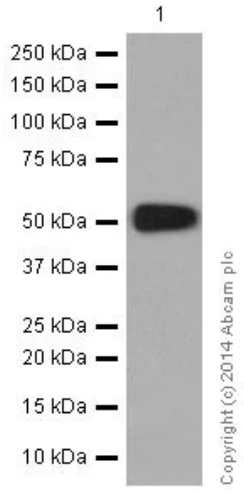
Lane 4:
PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysates at 10 µg

Lane 5:
NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysates at 10 µg

Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 50 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining of Rat brain lysates using rabbit Anti-alpha Tubulin antibody

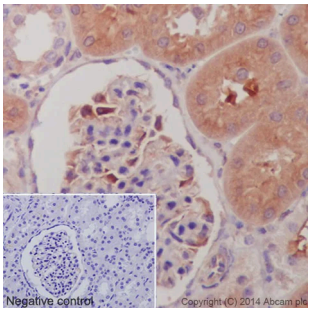
All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/50000 dilution

All lanes:
Rat brain lysates at 10 µg

Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 50 kDa

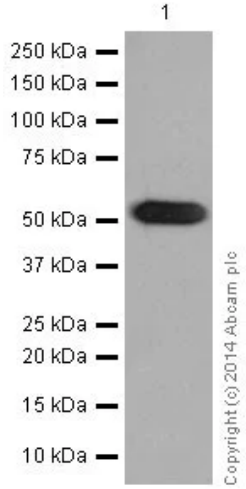


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

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

Immunohistochemistry analysis of paraffin-embedded Pig kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Pig kidney tubule and weak on glomerulus shown. Anti-Rabbit HRP (ab97051) used at a 1/100 dilution. Counter stained with Hematoxylin.
Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (ab97051) at 1/100 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining of Human fetal kidney lysates using rabbit Anti-alpha Tubulin antibody

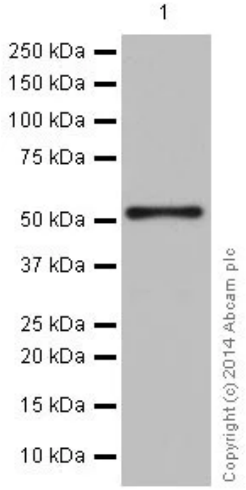
All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/50000 dilution

All lanes:
Human fetal kidney lysates at 10 µg

Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 50 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866)

Alpha Tubulin Western blot staining of Pig skeletal muscle lysates using rabbit Anti-alpha Tubulin antibody

All lanes:
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Loading Control (ab52866) at 1/5000 dilution

All lanes:
Pig skeletal muscle lysates at 20 µg

Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 50 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.