abcam

ab8245

Anti-GAPDH antibody [6C5] - Loading Control

Mouse anti-GAPDH antibody 6C5 ab8245 is a mouse monoclonal antibody that is used in GAPDH western blotting and immunofluorescence. Suitable for human, mouse and rat samples.

Most widely cited GAPDH monoclonal antibody clone on the market
Tried and trusted by researchers since 2002
Anti-GAPDH antibody clone 6C5 is cited in over 14560 publications
Same trusted quality, New lower price!

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.09% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Native Full Length Protein corresponding to Rabbit GAPDH. Database link P46406
Clone number	6C5
Purification technique	Affinity purification Protein A
Specificity	This GAPDH antibody can be used as a loading control antibody. GAPDH is a 146 kDa tetramer composed of four 30-40 kDa subunits. There is no cross-reaction with GAPDH from yeast. Preliminary data indicates that the GAPDH antibody-loading control ab8245 recognizes the monomer (36 kDa) and also the dimer forms of GAPDH, but not the tetrameric form of the protein.
Concentration	2 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Chromatography on protein A Sepharose

Reactivity data

WB

Tested

Species	Human
Dilution info	1/500.00000 - 1/10000.00000
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Saccharomyces cerevisiae, Cow, Goat, Horse, Chicken, Guinea pig, Hamster, Cat, Dog, Pig, Xenopus laevis, Fish, Monkey, Zebrafish, Baboon
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1.00000-5.00000 µg/mL
Notes	-

Species	Rat
Dilution info	1.00000-5.00000 µg/mL
Notes	-

Species	Human
Dilution info	1.00000-5.00000 µg/mL
Notes	-

Not recommended

Species	Saccharomyces cerevisiae, Cow, Goat, Horse, Chicken, Guinea pig, Hamster, Cat, Dog, Pig, Xenopus laevis, Fish, Monkey, Zebrafish, Baboon
Dilution info	-
Notes	-

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
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 switched from ascites to tissue culture supernatant on 31 July 2017. Lot numbers higher than [GR291713] will be from tissue culture supernatant.

Abcam is leading the way to address reproducibility in scientific research with our highly validated recombinant monoclonal and recombinant multiclonal antibodies. Search & select one of Abcam's thousands of recombinant alternatives to eliminate batch-variability and unnecessary animal use.

If you do not find a host species to meet your needs, our catalogue and custom Chimeric range provides scientists the specificity of Abcam's RabMAbs in the species backbone of your choice. Remember to also review our range of edited cell lines, proteins and biochemicals relevant to your target that may help you further your research goals.

Abcam antibodies are extensively validated in a wide range of species and applications, so please check the reagent specifications meet your scientific needs before purchasing. If you have any questions or bespoke requirements,

simply visit the Contact Us page to send us an inquiry or contact our Support Team ahead of purchase.

Supplementary info

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

Activity summary	GAPDH also known as glyceraldehyde 3-phosphate dehydrogenase plays a mechanical role in the glycolytic pathway where it catalyzes the sixth step converting glyceraldehyde 3-phosphate into 13-bisphosphoglycerate. This enzyme has a molecular weight of about 36 kDa. GAPDH is ubiquitously expressed in many tissues and cells making it an extensively studied protein in various biological processes. Due to its consistent expression level researchers often use GAPDH as a loading control in western blot experiments to ensure equal protein loading across samples.
Biological function summary	Glyceraldehyde 3-phosphate dehydrogenase contributes not only to energy production through glycolysis but also has roles beyond metabolism. It connects to cellular functions such as apoptosis and acts as a co-factor in RNA binding. Although it is not typically part of a stable protein complex its involvement in numerous cellular functions highlights its importance in maintaining cellular homeostasis.
Pathways	Glyceraldehyde 3-phosphate dehydrogenase integrates into glycolysis the central metabolic pathway for energy production in cells. Besides glycolysis it links to the regulation of apoptosis working alongside proteins like Bcl-2 which modulate cell survival. These pathways demonstrate the protein's critical role in balancing cell energy requirements and programmed cell death.
Associated diseases and disorders	Abnormalities in GAPDH expression and function relate to neurodegenerative conditions such as Alzheimer's disease and cancer. In Alzheimer's disease GAPDH interactions with proteins like amyloid-beta and tau proteins exacerbate neuronal damage. When overexpressed or dysfunctional in cancer GAPDH supports rapid cancer cell growth and proliferation by enhancing glycolytic flux a behavior known as the Warburg effect.

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.

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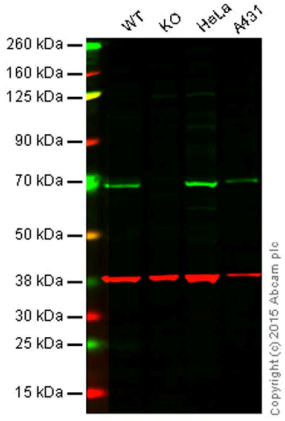
37 product images



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

This blot was produced using a 4-12% Bis-tris gel under the MES 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 before being incubated with ab140751 overnight at 4°C. Antibody binding was detected using the Donkey Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed ab216779 at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

All lanes:
Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 1/2000 dilution

Lane 1:
Wild-type HAP1 cell lysate (20 µg)

Lane 2:
NF-κB p60 knockout HAP1 cell lysate (20 µg)

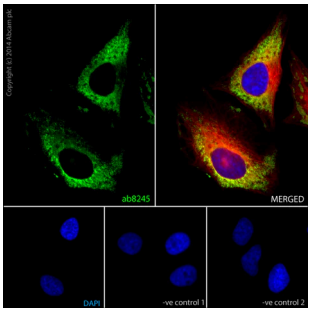
Lane 3:
HeLa cell lysate (20 µg)

Lane 4:
A431 cell lysate (20 µg)

Secondary

All lanes:
Western blot - Donkey Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216779) at 1/10000 dilution

Predicted band size: 36 kDa



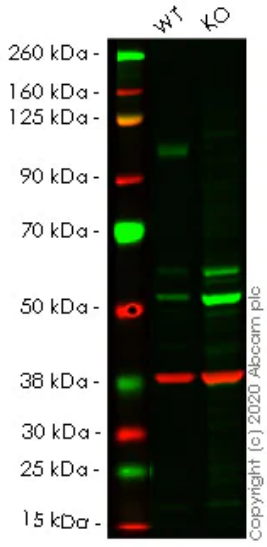
Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

GAPDH immunofluorescence staining of HeLa cells using mouse anti-GAPDH antibody

ab8245 staining GAPDH in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed with 100% methanol (5 minutes) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab8245 at 5 µg/ml and ab6046 at 1 µg/ml overnight at +4°C followed by a further incubation at room temperature for 1 hour with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) at 2 µg/ml (shown in green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (ab150088) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labeled 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.



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Lanes 1- 2: Merged signal (red and green). Green - ab16640 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab16640 was shown to react with Sortilin/NT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264772 (knockout cell lysate ab257696) was used. Wild-type HeLa and SORT1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab16640 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at a 1 µg/ml and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Sortilin/NT3 antibody (ab16640) at 1 µg/mL

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

Lane 2:
Western blot - Human SORT1 (Sortilin/NT3) knockout HeLa cell lysate (ab257696) at 20 µg

Secondary

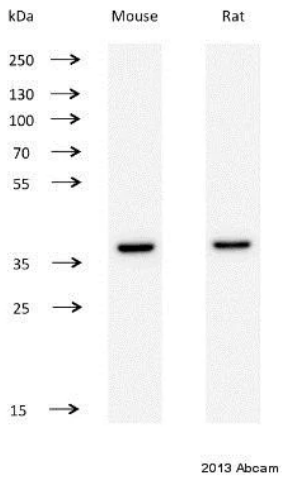
Lanes 1 - 2:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 92 kDa

Observed band size: 100 kDa



This image is courtesy of an anonymous customer review

Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

All lanes:
Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Lane 1:
Mouse hippocampus whole cell lysate at 20 µg

Lane 2:
Rat hippocampus whole cell lysate at 20 µg

Secondary

All lanes:
HRP-conjugated Rabbit anti-mouse at 1/5000 dilution

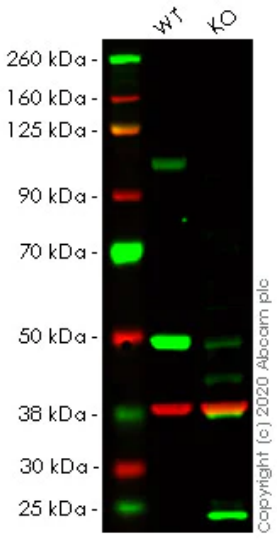
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 36 kDa

Observed band size: 36 kDa

Exposure time: 10s



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: SORT1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - ab188586 observed at 100 kDa. Red - loading control ab8245 observed at 37 kDa.

ab188586 Anti-Sortilin/NT3 antibody [EPR15010] was shown to specifically react with Sortilin/NT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264772 (knockout cell lysate ab257696) was used. Wild-type and Sortilin/NT3 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab188586 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4˚C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Sortilin/NT3 antibody [EPR15010] (ab188586) at 1/1000 dilution

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

Lane 2:
Western blot - Human SORT1 (Sortilin/NT3) knockout HeLa cell lysate (ab257696) at 20 µg

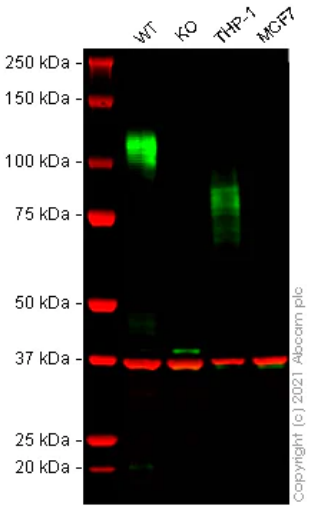
Secondary

Lanes 1 - 2:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution
Performed under reducing conditions.

Predicted band size: 92 kDa

Observed band size: 100 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

False colour image of Western blot: Anti-SIRPA antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, the antibody was shown to bind specifically to SIRPA. A band was observed at 100-140 kDa (mouse SIRPA, isoform 1), in wild-type RAW 264.7 cell lysates (band observed at 70-100 kDa in THP-1 is Human SIRPA) with no signal observed at this size in SIRPA knockout cell line ab281618 (knockout cell lysate ab282969). To generate this image, wild-type and SIRPA knockout RAW 264.7 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

All lanes:
Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 1/20000 dilution

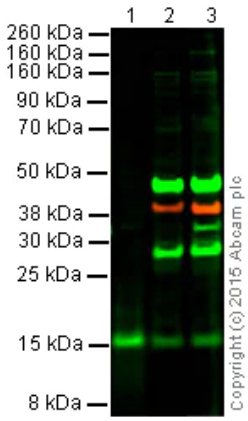
Lane 1:
Wild-type RAW 264.7 cell lysate at 20 µg

Lane 2:
RAW 264.7 cell lysate at 20 µg

Lane 3:
THP-1 cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Predicted band size: 55 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 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 unpurified ab108319 (1/1000) overnight at 4°C. ab8245 (mouse anti-GAPDH; 0.05 ug/mL) was included as a loading control. Antibody binding was detected using goat anti-rabbit IgG IR-680 (green) and goat anti-mouse IgG IR800 (red) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx

All lanes:
Western blot - Anti-BDNF antibody [EPR1292] (ab108319) at 1/1000 dilution

Lane 1:
Human hippocampus lysate at 20 µg

Lane 2:
Rat hippocampus lysate at 20 µg

Lane 3:
Mouse hippocampus lysate at 20 µg

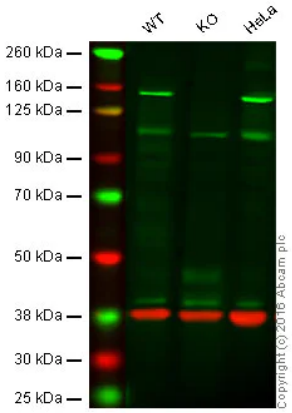
Secondary

All lanes:
Gt anti Rb IR680 at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 27 kDa

Observed band size: 15 kDa, 28 kDa, 35 kDa, 45 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

This blot was produced using a 3-8% Tris Acetate gel under the TA 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 before being incubated with ab128874 overnight at 4°C. Antibody binding was detected using the Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ab216773. Loading control to GAPDH ab8245 antibody binding was detected using the Goat anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

Merged signal (red and green). Green Anti-Brd4 antibody [EPR5150(2)] ab128874 observed at 150 kDa using Goat anti-Rabbit IgG H&L (IRDye® 800CW)-ab216773 as secondary antibody. Red - Anti-GAPDH antibody loading control, ab8245, observed at 37 kDa,

Lane 1:
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Lanes 1 - 3:
Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 1/10000 dilution

Lane 1:
Wild-type HAP1 cell lysate (20 µg)

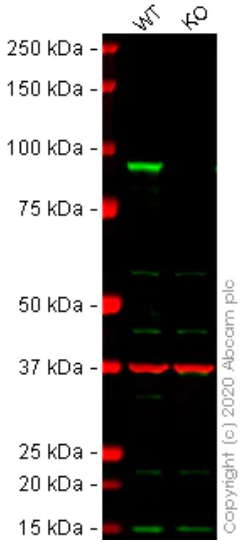
Lane 2:

Brd4 knockout HAP1 cell lysate (20 µg)

Lane 3:
HeLa cell lysate (20 µg)

Secondary

All lanes:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/10000 dilution
Predicted band size: 152 kDa, 36 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Lane 1: Wild-type HCT116 cell lysate 20 ug
Lane 2: CTNNB1 knockout HCT116 cell lysate 20 ug
Lanes 1 - 2: Merged signal (red and green). Green - ab223075 observed at 95 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab223075 was shown to react with Anti-beta Catenin in wild-type HCT 116 cells in western blot with loss of signal observed in CTNNB1 knockout cell line ab273712 (CTNNB1 knockout cell lysate ab275247). HCT 116 wild-type and CTNNB1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluoroscent western blot (TBS-based) blocking solution 50% (v/v) in TBS-T (0.1% Tween®) before incubation with ab223075 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4˚C at 1 ug/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-beta Catenin antibody [IGX4794R-3] (ab223075) at 1 µg/mL

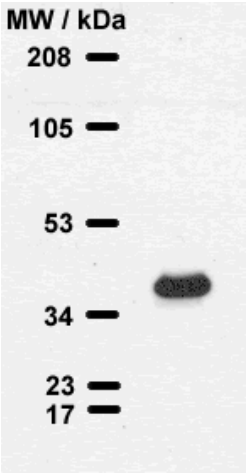
Lane 1:
Wild-type HCT116 cell lysate at 20 µg

Lane 2:
CTNNB1 knockout HCT116 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 95 kDa

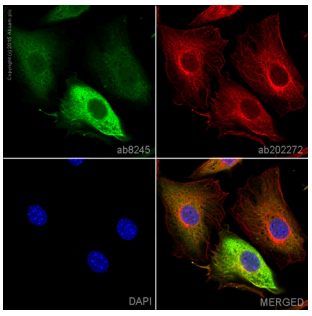


Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

All lanes:
Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 10 µg/mL

All lanes:
Raji (Human Burkitt's lymphoma cell line) whole cell lysate at 20 µg

Predicted band size: 36 kDa



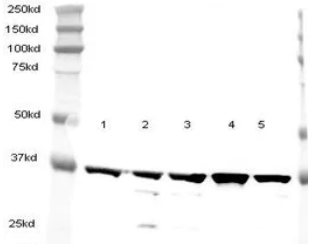
Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

GAPDH immunofluorescence staining of 3T3 cells using mouse anti-GAPDH antibody

ab8245 staining GAPDH in NIH/3T3 (Mouse embryo fibroblast cell line) cells.

The cells were fixed with 4% formaldehyde (10 minutes) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab8245 at 1 µg/ml and ab202272 at 1/250 dilution overnight at +4°C followed by a further incubation at room temperature for 1 hour with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) (shown in green). Nuclear DNA was labeled in blue with DAPI.

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



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Fluorescence detection of secondary antibody.

All lanes:
Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 2.5 µg/mL

Lane 1:
HeLa (Human epithelial cell line from cervix adenocarcinoma) Nuclear at 20 µg

Lane 2:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 3:
A431 (Human epidermoid carcinoma cell line) cell lysate at 20 µg

Lane 4:
Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate at 20 µg

Lane 5:
HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate at 20 µg

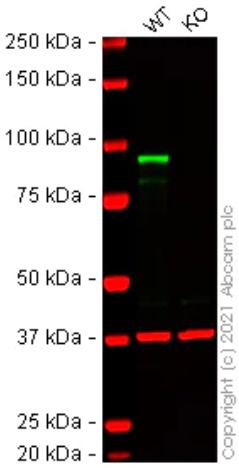
Secondary

All lanes:
Alexa Fluor anti-mouse at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 36 kDa

Observed band size: 37 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Lane 1: Wild-type HCT 116 cell lysate 20 µg
Lane 2: CTNNB1 knockout HCT 116 cell lysate 20 µg
False colour image of Western blot: Anti-beta Catenin antibody [IGX4794R-3] staining at 1 µg/ml, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab223075 was shown to bind specifically to beta Catenin. A band was observed at 95 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CTNNB1 knockout cell line ab273712 (knockout cell lysate ab275247). The band observed in the knockout lysate lane below 95 kDa is likely to represent a truncated form of beta Catenin. 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 CTNNB1 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

All lanes:
Western blot - Anti-beta Catenin antibody [IGX4794R-3] (ab223075) at 1 µg/mL

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

Lane 2:
CTNNB1 knockout HCT 116 cell lysate at 20 µg

Secondary

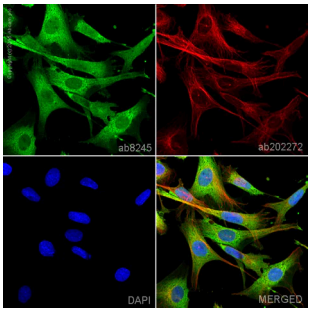
Lanes 1 - 2:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 95 kDa



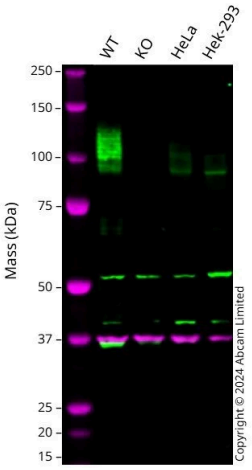
Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

GAPDH immunofluorescence staining of SC40LT-SMC cells using mouse anti-GAPDH antibody

ab8245 staining GAPDH in SV40LT-SMC (Rat SV40-transfected aorta smooth cell line) cells.

The cells were fixed with 4% formaldehyde (10 minutes) permeabilized with 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 1 hour. The cells were then incubated with ab8245 at 5µg/ml and ab202272 at 1/250 dilution overnight at +4°C followed by a further incubation at room temperature for 1h with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) (shown in green). Nuclear DNA was labeled in blue with DAPI.

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



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-SLC26A2 antibody [EPR27119-17] (ab308625) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab308625 was shown to bind specifically to SLC26A2. A band was observed at 82 kDa in wild-type A549 cell lysates with no signal observed at this size in SLC26A2 knockout cell line. To generate this image, wild-type and SLC26A2 knockout A549 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution. For optimal results, we recommend using fluorescent western blot (TBS-based) blocking solution and not boiling the samples.

All lanes:
Western blot - Anti-SLC26A2/DTD antibody [EPR27119-17] (ab308625) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
SLC26A2 knockout A549 cell lysate at 20 µg

Lane 3:
HeLa UNBOILED cell lysate at 20 µg

Lane 4:
HEK-293 UNBOILED cell lysate at 20 µg

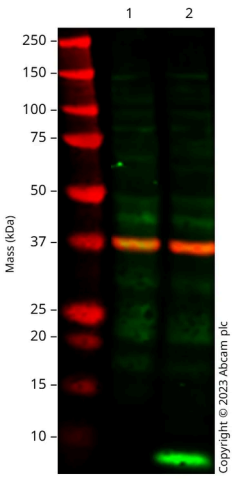
Secondary

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

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

Performed under reducing conditions.

Observed band size: 82 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Rabbit Monoclonal to SARS-CoV2 Envelope Protein (ab320094) staining at 1000 dilution, shown in Green; Mouse anti-GAPDH antibody [6C5] (ab8245) at 1/20000 dilution, shown in red.

In Western blot, ab320094 was shown to bind specifically to SARS-CoV2 Envelope protein. A band was observed at 8 kDa in HEK-293T overexpressing SARS-CoV2 Envelope cell lysates with no signal observed at this size in HEK-293T mock transfected cell lysate. 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-SARS-CoV2 envelope protein antibody [EPR24857-260] (ab320094) at 1/1000 dilution

Lane 1:
HEK-293T mock transfected cell lysate at 20 µg

Lane 2:
HEK-293T overexpressing SARS-CoV2 Envelope cell lysate at 20 µg

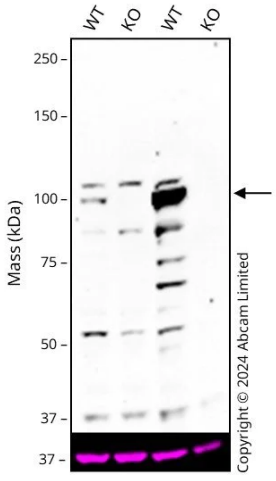
Secondary

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

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

Performed under reducing conditions.

Observed band size: 8 kDa, 37 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-BRD3 antibody [EPR23743-226] (ab300106) staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab300106 was shown to bind specifically to BRD3. A band was observed at 90-100 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in BRD3 knockout cell line. To generate this image, wild-type and BRD3 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 HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-BRD3 antibody [EPR23743-226] (ab300106) at 1/500 dilution

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

Lane 2:
BRD3 knockout HCT 116 cell lysate at 20 µg

Lane 3:
Wild-type HEK-293T ab255553 cell lysate at 20 µg

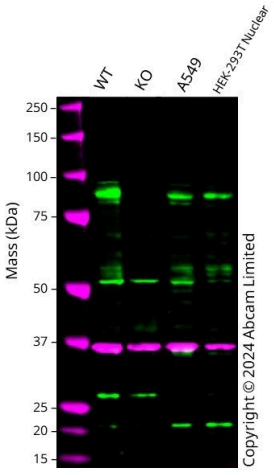
Lane 4:
BRD3 knockout HEK-293T ab260529 cell lysate at 20 µg

Secondary

Lanes 1 - 4:
HRP conjugated Goat anti-Rabbit (H+L) at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution
Performed under reducing conditions.

Observed band size: 90-100 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-UVRAG antibody [EPR27025-83] (ab313626) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab313626 was shown to bind specifically to UVRAG. A band was observed at 78-100 kDa in wild-type MCF7 cell lysates with no signal observed at this size in UVRAG knockout cell line. To generate this image, wild-type and UVRAG knockout MCF7 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-UVRAG antibody [EPR27025-83] (ab313626) at 1/1000 dilution

Lane 1:
Wild-type MCF7 cell lysate at 20 µg

Lane 2:
UVRAG knockout MCF7 cell lysate at 20 µg

Lane 3:
A549 cell lysate at 20 µg

Lane 4:
HEK-293T Nuclear cell lysate at 20 µg

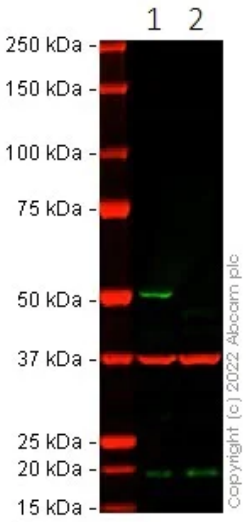
Secondary

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

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

Performed under reducing conditions.

Observed band size: 78-100 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

False colour image of Western blot: Anti-Smad3 antibody [EP568Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40854 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type A549 cell lysates with no signal observed at this size in SMAD3 CRISPR-Cas9 edited cell line ab277888 (CRISPR-Cas9 edited cell lysate None). The band observed in the CRISPR-Cas9 edited lysate lane below 50 kDa is likely to represent a truncated form of Smad3. 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 SMAD3 CRISPR-Cas9 edited A549 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Smad3 antibody [EP568Y] (ab40854) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
SMAD3 CRISPR-Cas9 edited A549 cell lysate at 20 µg

Secondary

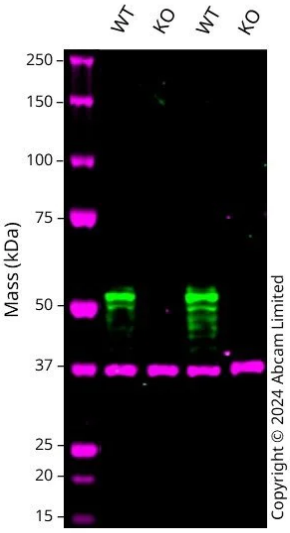
Lanes 1 - 2:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

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

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 50 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-STK11 antibody [EPR19379] (ab199970) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab199970 was shown to bind specifically to STK11. A band was observed at 50-60 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in STK11 knockout cell line. To generate this image, wild-type and STK11 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-LKB1 antibody [EPR19379] (ab199970) at 1/1000 dilution

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

Lane 2:
STK11 knockout HCT 116 cell lysate at 20 µg

Lane 3:
Wild-type HEK-293T ab255553 cell lysate at 20 µg

Lane 4:
STK11 knockout HEK-293T ab261047 cell lysate at 20 µg

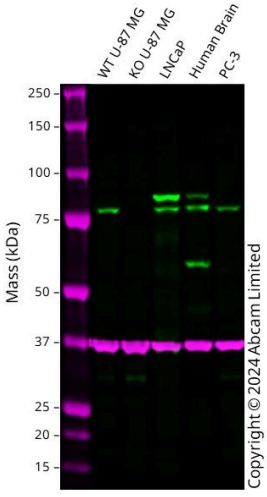
Secondary

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

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

Performed under reducing conditions.

Observed band size: 50-60 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Rabbit Monoclonal[EPR23994-103] to Calcium-independent Phospholipase A2/PLA2G6 ab259950 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 76 kDa in Wild-type U-87 MG cell lysates with no signal observed at this size in PLA2G6 knockout U-87 MG 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-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-Calcium-independent Phospholipase A2/PLA2G6 antibody [EPR23994-103] (ab259950) at 1/1000 dilution

Lane 1:
Wild-type U-87 MG at 20 µg

Lane 2:
Western blot - Human PLA2G6 knockout U-87 MG cell line (ab306752) at 20 µg

Lane 3:
LNCaP at 20 µg

Lane 4:
Human Brain at 20 µg

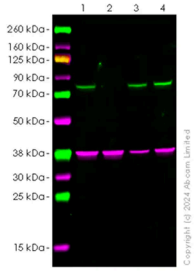
Lane 5:
PC-3 at 20 µg

Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution
Performed under reducing conditions.

Predicted band size: 90 kDa

Observed band size: 76 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of TBS.

The samples were run on a Bis-Tris gel under reducing conditions.

Western blot: Anti-MIRO2 antibody (ab) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta.

In Western blot, ab was shown to bind specifically to MIRO2. Target of interest was observed at 80 kDa in wild-type HeLa cell lysates (lane 1) with no signal observed at this size MIRO2 knockout cell line (lane 2, knockout cell line ab265801 / knockout cell lysate ab257639). To generate this image, samples were first run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in a fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed in TBS-T, incubated with secondary antibodies Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution for 1 h at room temperature, washed again then imaged.

All lanes:
Western blot - Anti-MIRO2 antibody [EPR29118-10] (ab321803) at 1/1000 dilution

Lane 1:
Wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
MIRO2 knockout HeLa whole cell lysate at 20 µg

Lane 3:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

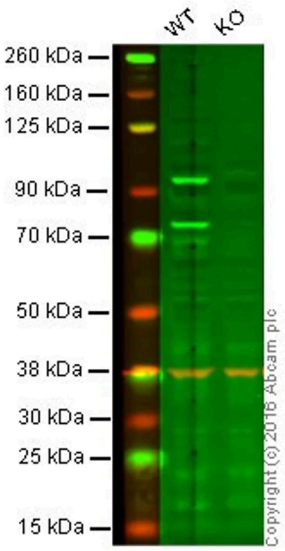
Lane 4:

K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (800CW) and Goat Anti-Mouse IgG H&L (680RD) at 1/20000 dilution
Performed under reducing conditions.

Observed band size: 80 kDa, 36 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Lanes 1 - 2: Merged signal (red and green). Green - ab108507 observed at 70 kDa. Red - loading control, ab8245, observed at 37 kDa.

In Western blot: ab108507 was shown to specifically react with LIM Kinase 1 when LIM Kinase 1 knockout samples were used. Wild-type and LIM Kinase 1 knockout samples were subjected to SDS-PAGE. ab108507 and ab8245 (loading control to GAPDH) were diluted at 1/500 and 1/10000 dilution respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®

800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®

680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-LIM Kinase 1 antibody [EPR912] (ab108507) at 1/500 dilution

Lane 1:
Wild-type HAP1 cell lysate at 40 µg

Lane 2:
LIMK1 knockout HAP1 cell lysate at 40 µg

Secondary

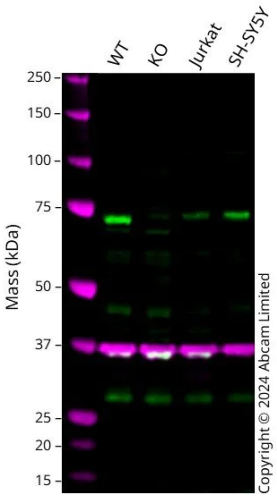
Lanes 1 - 2:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 73 kDa

Observed band size: 70 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-COIL antibody (ab210785) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab210785 was shown to bind specifically to COIL. A band was observed at 68 kDa in wild-type HeLa cell lysates with no signal observed at this size in COIL knockout cell line. To generate this image, wild-type and COIL knockout HeLa 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Coilin antibody (ab210785) at 1/1000 dilution

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

Lane 2:
COIL knockout HeLa cell lysate at 20 µg

Lane 3:
Jurkat cell lysate at 20 µg

Lane 4:
SH-SY5Y cell lysate at 20 µg

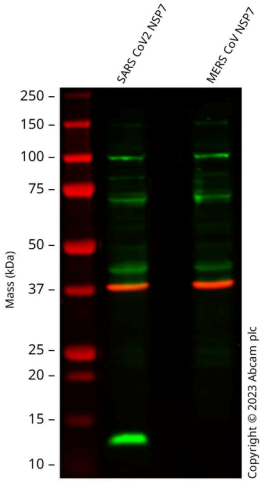
Secondary

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

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

Performed under reducing conditions.

Observed band size: 68 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Rabbit monoclonal to SARS-CoV2 nsp7 (ab320092) staining at 1000 dilution, shown in Green; Mouse anti-GAPDH antibody [6C5] (ab8245) at 1/20000 dilution, shown in red.

In Western blot, ab320092 was shown to bind specifically to SARS-CoV2 NSP7 protein. A band was observed at 15 kDa in HEK-293T transfected with SARS-CoV2 nsp7 cell lysates with no signal observed at this size in HEK-293T transfected with MERS-CoV nsp7. 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-SARS-CoV2 nsp7 antibody [EPR24846-6] (ab320092) at 1/1000 dilution

Lane 1:
HEK-293T transfected with SARS-CoV2 nsp7 protein cell lysate at 20 µg

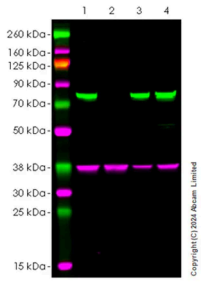
Lane 3:
HEK-293T transfected with MERS-CoV nsp7 protein cell lysate at 20 µg

Secondary

Lanes 1 and 3:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 and 3:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution
Performed under reducing conditions.

Observed band size: 15 kDa, 37 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Blocking buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of TBS.

Diluting buffer and concentration : Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBST

The samples were run on a Bis-Tris gel under reducing conditions.

Western blot: Anti-MIRO2 antibody (ab320739) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta.

In Western blot, ab320739 was shown to bind specifically to MIRO2. Target of interest was observed at 80 kDa in wild-type HeLa cell lysates (lane 1) with no signal observed at this size MIRO2 knockout cell line (lane 2, knockout cell line ab265801 / knockout cell lysate ab257639). To generate this image, samples were first run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in a fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed in TBS-T, incubated with secondary antibodies Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution for 1 h at room temperature, washed again then imaged.

Exposure time: N/A

All lanes:
Western blot - Anti-MIRO2 antibody [EPR29118-85] (ab320739) at 1/1000 dilution

Lane 1:
Wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Western blot - Human RHOT2 (MIRO2) knockout HeLa cell lysate (ab257639) at 20 µg

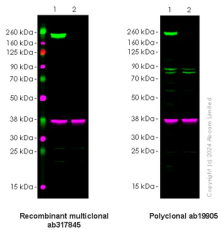
Lane 3:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 4:
K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (800CW) and Goat Anti-Mouse IgG H&L (680RD) at 1/20000 dilution
Performed under reducing conditions.

Observed band size: 80 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of TBS.

Lysates at 20 µg per lane.

The samples were run on a Bis-Tris gel under reducing conditions.

Western blot: Anti-Dnmt1 antibody (ab317845) staining at 1/1000 dilution and Anti-Dnmt1 antibody (ab19905) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5](ab8245) loading control staining at 1/20000 dilution, shown in magenta.

In Western blot, ab317845 was shown to bind specifically to Dnmt1. Target of interest was observed at 183-184 kDa in wild-type HAP1 cell lysates (lane 1) with no signal observed at this size in Dnmt1 knockout cell line (lane 2). To generate this image, samples were first run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in a fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed in TBS-T, incubated with secondary antibodies Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution for 1 h at room temperature, washed again then imaged.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 31426844).

All lanes:
Western blot - Anti-Dnmt1 antibody [RM1192] (ab317845) at 1/1000 dilution

Lane 1:
Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 20 µg

Lane 2:
Dnmt1 knockout HAP1 whole cell lysate at 20 µg

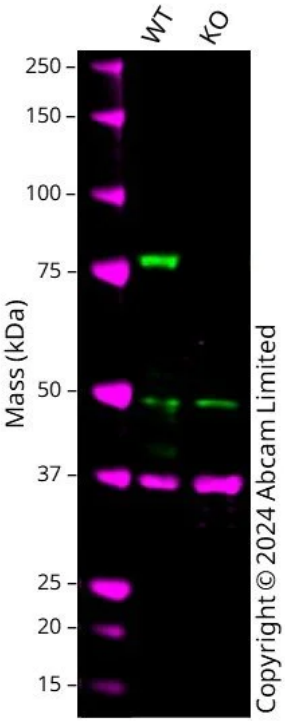
Secondary

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

Performed under reducing conditions.

Observed band size: 183 kDa, 184 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-PRKCD antibody [EPR17075] (ab182126) staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab182126 was shown to bind specifically to PRKCD. A band was observed at 75-80 kDa in wild-type U-87 MG cell lysates with no signal observed at this size in PRKCD knockout cell line. To generate this image, wild-type and PRKCD knockout U-87 MG 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-PKC delta antibody [EPR17075] (ab182126) at 1/5000 dilution

Lane 1:
Wild-type U-87 MG cell lysate at 20 µg

Lane 2:
PRKCD knockout U-87 MG cell lysate at 20 µg

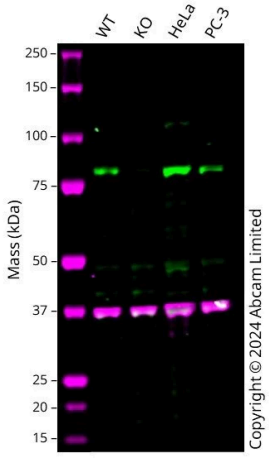
Secondary

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

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

Performed under reducing conditions.

Observed band size: 75-80 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-MFN2 antibody [NIAR164] (ab124773) staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab124773 was shown to bind specifically to MFN2. A band was observed at 64 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in MFN2 knockout cell line. To generate this image, wild-type and MFN2 knockout HEK-293 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Mitofusin 2 antibody [NIAR164] (ab124773) at 1/5000 dilution

Lane 1:
Wild-type HEK-293 cell lysate at 20 µg

Lane 2:
MFN2 knockout HEK-293 cell lysate at 20 µg

Lane 3:
HeLa cell lysate at 20 µg

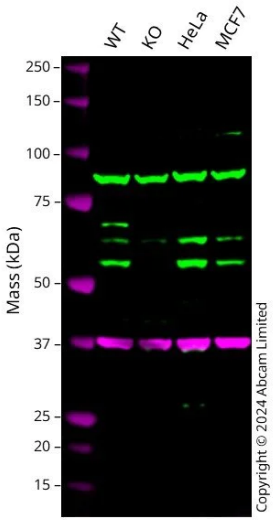
Lane 4:
PC-3 cell lysate at 20 µg

Secondary

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

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution
Performed under reducing conditions.

Observed band size: 64 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-PUS3 antibody (ab211270) staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab211270 was shown to bind specifically to PUS3. A band was observed at 54-60 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in PUS3 knockout cell line. To generate this image, wild-type and PUS3 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 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-PUS3 antibody - C-terminal (ab211270) at 1/500 dilution

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

Lane 2:
PUS3 knockout HCT 116 cell lysate at 20 µg

Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

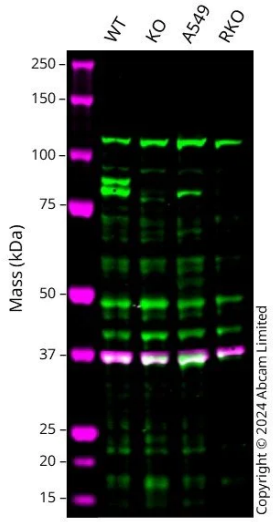
Secondary

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

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

Performed under reducing conditions.

Observed band size: 54-60 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-SEMA3B antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, anti-SEMA3B antibody was shown to bind specifically to SEMA3B. A band was observed at 83 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in SEMA3B knockout cell line. To generate this image, wild-type and SEMA3B 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:

Anti-SEMA3B antibody at 1/1000 dilution

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

Lane 2:
SEMA3B knockout HCT 116 cell lysate at 20 µg

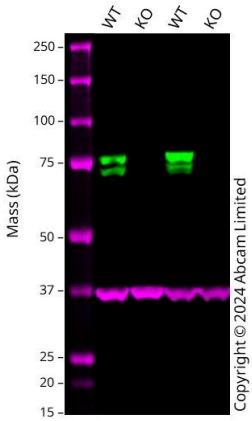
Lane 3:
A549 cell lysate at 20 µg

Lane 4:
RKO cell lysate at 20 µg

Secondary

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

Observed band size: 83 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-FMRP antibody ab17722 staining at 1 µg/mL, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 75 kDa in Wild-type U-87 MG cell lysates with no signal observed at this size in FMR1 knockout U-87 MG cell line. To generate this image, 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-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-FMRP antibody (ab17722) at 1 µg/mL

Lane 1:
Wild-type U-87 MG at 20 µg

Lane 2:
Western blot - Human FMR1 knockout U-87 MG cell line (ab306664) at 20 µg

Lane 3:
Wild-type A549 at 20 µg

Lane 4:
Western blot - Human FMR1 knockout A549 cell line (ab288956) at 20 µg

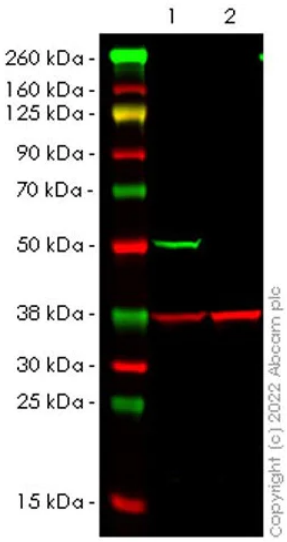
Secondary

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

Performed under reducing conditions.

Predicted band size: 71 kDa

Observed band size: 75 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Blocking buffer and concentration: ½ volume of Odyssey Blocking Buffer (TBS)+ ½ volume of 0.1% TBS Diluting buffer and concentration: ½ volume of Odyssey Blocking Buffer (TBS)+ ½ volume of 0.1% TBST

False colour image of Western blot: Anti-Coronin-1C antibody [EPR25365-24] (ab283693) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab283693 was shown to bind specifically to Coronin-1C. A band was observed at 53 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in Coronin-1C knockout cell line ab266381 (knockout cell lysate (ab258377).

To generate this image, wild-type and Coronin-1C knockout HEK-293T cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

All lanes:
Western blot - Anti-Coronin-1C antibody [EPR25365-24] (ab283693) at 1/1000 dilution

Lane 1:
Wild-type 293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
Western blot - Human CORO1C (Coronin-1C) knockout HEK-293T cell lysate (ab258377) at 20 µg

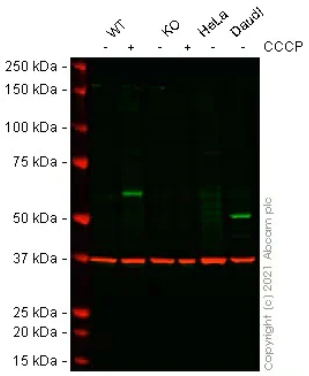
Secondary

Lanes 1 - 2:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Lanes 1 - 2:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

False colour image of Western blot: Anti-PINK1 antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, the antibody was shown to bind specifically to PINK1. A band was observed at 63 kDa in wild-type cell lysates with no signal observed at this size in PINK1 knockout cell line ab266393 (knockout cell lysate ab257030). To generate this image, wild-type and PINK1 knockout 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

All lanes:
Anti-PINK1 antibody at 1/1000 dilution

Lane 1:
Wild-type HEK-293T Vehicle Control CCCP (0 µM, 24 h) cell lysate at 20 µg

Lane 2:
Wild-type HEK-293T Treated CCCP (10 µM, 24 h) cell lysate at 20 µg

Lane 3:
PINK1 knockout HEK-293T Vehicle Control CCCP (0 µM, 24 h) cell lysate at 20 µg

Lane 4:
PINK1 knockout HEK-293T Treated CCCP (10 µM, 24 h) cell lysate at 20 µg

Lane 5:
HeLa cell lysate at 20 µg

Lane 6:
Daudi cell lysate at 20 µg

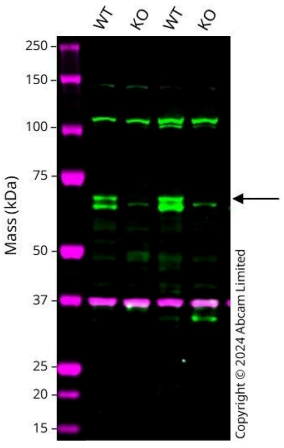
Secondary

Lanes 1 - 6:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Lanes 1 - 6:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 63 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Western blot: Anti-KEAP1 antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, anti-KEAP1 antibody was shown to bind specifically to KEAP1. A band was observed at 70 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in KEAP1 knockout cell line. To generate this image, wild-type and KEAP1 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Anti-KEAP1 antibody at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
KEAP1 knockout A549 cell lysate at 20 µg

Lane 3:
Wild-type HCT 116 cell lysate at 20 µg

Lane 4:
KEAP1 knockout HCT 116 ab286484 cell lysate at 20 µg

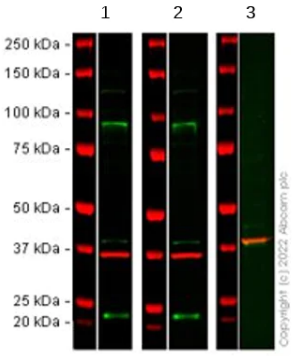
Secondary

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

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

Performed under reducing conditions.

Observed band size: 70 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

ab287893 was shown to specifically react with EZH2 (pT345) in HEK-293. The signal was significantly reduced after blocking with 1µg/ml histone-lysine N-methyltransferase EZH2 peptide (pT345). Samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. ab287893 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilutions, respectively. Blots were developed with Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-EZH2 antibody [EPR24902-112] (ab287893) at 1/1000 dilution

Lane 1:
HEK-293 cell lysate at 20 µg

Lane 2:
HEK-293 cell lysate with histone-lysine N-methyltransferase EZH2 peptide (unmodified) at 1µg/ml at 20 µg

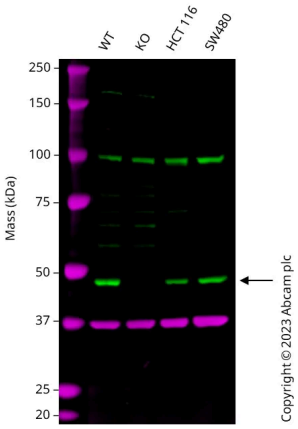
Lane 3:
HEK-293 cell lysate with histone-lysine N-methyltransferase EZH2 peptide (pT345) at 1µg/ml at 20 µg

Secondary

Lanes 1 - 3:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 3:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution
Performed under reducing conditions.

Observed band size: 98 kDa, 37 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Anti-TRIP13 antibody (ab128171) staining at 1/2000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab128171 was shown to bind specifically to TRIP13. A band was observed at 48 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in TRIP13 knockout cell line. To generate this image, wild-type and TRIP13 knockout HEK-293T 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-TRIP13/PCH2 antibody (ab128171) at 1/2000 dilution

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
TRIP13 knockout HEK-293T cell lysate at 20 µg

Lane 3:
HCT 116 cell lysate at 20 µg

Lane 4:
SW480 cell lysate at 20 µg

Predicted band size: 49 kDa

Observed band size: 48 kDa