abcam

ab55271

Anti-IDH2 antibody [5F11]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human IDH2 aa 350-500. The exact immunogen used to generate this antibody is proprietary information. Database link P48735
Clone number	5F11
Purity	Tissue culture supernatant
Light chain type	kappa

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant fragment - Human
Dilution info	-
Notes	This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein.

Species	Human
Dilution info	-
Notes	This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein.

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Target data

See full target information IDH2

Function	Plays a role in intermediary metabolism and energy production (PubMed:19228619, PubMed:22416140). It may tightly associate or interact with the pyruvate dehydrogenase complex (PubMed:19228619, PubMed:22416140).

Storage

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

Notes

This product was changed from ascites to tissue culture supernatant on 13th Feb 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

Supplementary info

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

Activity summary	Isocitrate dehydrogenase 2 (IDH2) is an enzyme that converts isocitrate to alpha-ketoglutarate in the citric acid cycle through oxidative decarboxylation. IDH2 also known as NADP-dependent isocitrate dehydrogenase has a molecular weight of about 51 kDa. This protein expresses in the mitochondria serving an important role in cellular energy production and intermediary metabolism. By facilitating this conversion IDH2 impacts cellular respiration and energy balance.
Biological function summary	The IDH2 enzyme facilitates cellular metabolism by producing NADPH critical for biosynthesis and antioxidant defense. It functions within the oxidative decarboxylation of isocitrate providing reducing equivalents to keep cellular redox balance. Although not part of a larger enzymatic complex IDH2 acts synergistically with other metabolic enzymes to maintain cellular biochemical pathways.
Pathways	The IDH2 protein participates in the citric acid cycle and redox homeostasis. IDH2 contributes to the tricarboxylic acid (TCA) pathway coupling with other TCA components such as citrate synthase and aconitase. Within the redox pathway it influences glucose metabolism via its link with enzymes like NADPH oxidase ensuring a steady supply of NADPH for biosynthetic reactions.
Associated diseases and disorders	The IDH2 protein links to certain cancers like acute myeloid leukemia (AML) due to mutations such as IDH2 R140Q and IDH2 R172K which lead to the production of oncometabolite 2-hydroxyglutarate. This oncometabolite influences epigenetic regulation and cellular differentiation. IDH2 mutations often associate with altered tumor suppressor pathways involving proteins like TP53 contributing to tumorigenesis and impaired cellular differentiation.

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

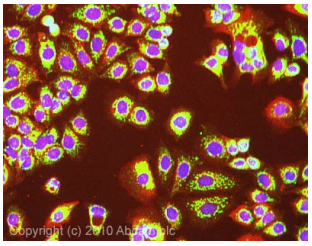
We do not recommend this combination. It is not covered by our product promise.

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.

7 product images

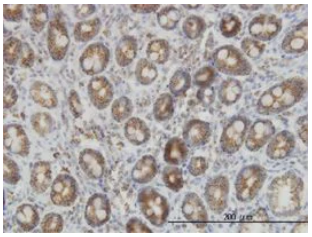


Immunocytochemistry/ Immunofluorescence - Anti-IDH2 antibody [5F11] (ab55271)

IDH2 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-IDH2 antibody

ICC/IF image of ab55271 stained Mcf7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab55271, 10µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

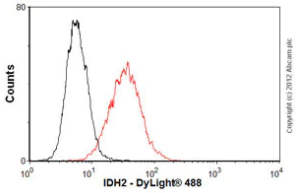
This image was generated using the ascites version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IDH2 antibody [5F11] (ab55271)

IDH2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-IDH2 antibody

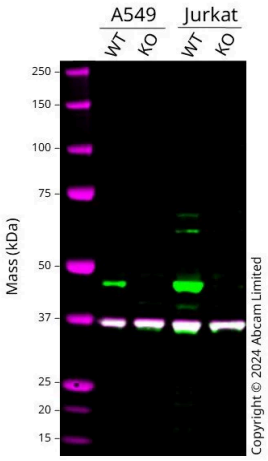
IDH2 antibody (ab55271) used in immunohistochemistry at 3ug/ml on formalin fixed and paraffin embedded human colon.
This image was generated using the ascites version of the product.



Flow Cytometry - Anti-IDH2 antibody [5F11] (ab55271)

IDH2 Flow Cytometry staining using mouse Anti-IDH2 antibody

Overlay histogram showing MCF7 cells stained with ab55271 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab55271, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in MCF7 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.
This image was generated using the ascites version of the product.



Western blot - Anti-IDH2 antibody [5F11] (ab55271)

IDH2 Western blot staining using mouse Anti-IDH2 antibody

Western blot: Anti-IDH2 antibody [5F11] (ab55271) staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab55271 was shown to bind specifically to IDH2. A band was observed at 51 kDa in wild-type A549 cell lysates with no signal observed at this size in IDH2 knockout cell line. To generate this image, wild-type and IDH2 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 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-IDH2 antibody [5F11] (ab55271) at 1/1000 dilution

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

Lane 2:
IDH2 knockout A549 cell lysate at 20 µg

Lane 3:
Wild-type Jurkat cell lysate at 20 µg

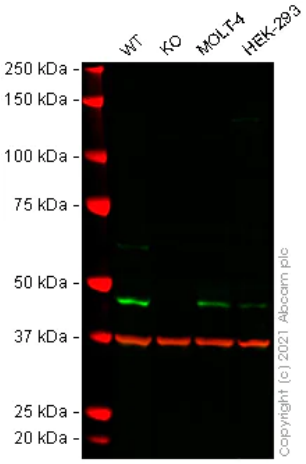
Lane 4:
IDH2 knockout Jurkat ab282331 cell lysate at 20 µg

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.

Observed band size: 51 kDa



Western blot - Anti-IDH2 antibody [5F11] (ab55271)

IDH2 Western blot staining using mouse Anti-IDH2 antibody

False colour image of Western blot: Anti-IDH2 antibody [5F11] staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab55271 was shown to bind specifically to IDH2. A band was observed at 48 kDa in wild-type Jurkat cell lysates with no signal observed at this size in IDH2 knockout cell line ab282331 (knockout cell lysate ab283148). To generate this image, wild-type and IDH2 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 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 (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.
All lanes:
Western blot - Anti-IDH2 antibody [5F11] (ab55271) at 1/1000 dilution

Lane 1:

Wild-type Jurkat cell lysate at 20 µg

Lane 2:
IDH2 knockout Jurkat cell lysate at 20 µg

Lane 2:
Western blot - Human IDH2 knockout Jurkat cell line (ab282331)

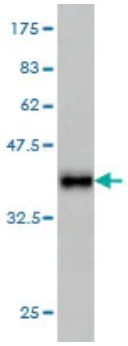
Lane 3:
MOLT-4 cell lysate at 20 µg

Lane 4:
HEK-293 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 48 kDa



Western blot - Anti-IDH2 antibody [5F11] (ab55271)

Western blot against tagged recombinant protein immunogen using ab55271 IDH2 antibody at 1ug/ml. Predicted band size of immunogen is 37 kDa
This image was generated using the ascites version of the product.

All lanes:
Western blot - Anti-IDH2 antibody [5F11] (ab55271)

Predicted band size: 50 kDa

i

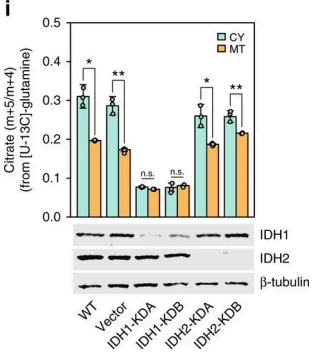


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Western blot - Anti-IDH2 antibody [5F11] (ab55271)

IDH2 Western blot staining using mouse Anti-IDH2 antibody

Western Blotting using Anti-IDH2 antibody [5F11], ab55271. Publication image from Lee, W. D. et al., 2019, Nat Commun, 30903027. Legend direct from paper.

A quantitative view of mitochondrial and cytosolic fluxes in the TCA cycle and citrate metabolism under normoxia. a–d Mass-isotopomer labeling kinetics of citrate in mitochondria (a) and cytosol (b), and malate in mitochondria (c) and cytosol (d) when feeding HeLa cells with [U-13C]-glutamine under standard normoxic conditions. e Measured isotopic labeling ratio in HeLa cells for citrate m + 5 /m + 4 and malate m + 3 /m + 4 in media (red) in comparison to the expected labeling via computational simulation, considering the measured labeling kinetics of citrate and malate in mitochondria (green) and cytosol (orange). f Gibbs free energy of mitochondrial (IDH2/3) and cytosolic (IDH1) IDH isozymes (in the oxidative direction) in HeLa cells under normoxia (green) and hypoxia (red). g Mitochondrial and cytosolic fluxes, showing percentage from citrate synthase flux (which is 0.48 mM h−1). Arrow represents the direction of net flux; number represents net flux in the direction of the arrow and number in parenthesis correspond to the backward flux. Confidence intervals for estimated fluxes are shown in Supplementary Data 8. h The measured mass-isotopomer distribution of palmitate when feeding cells with [U-13C]-glutamine (green) is consistent with the simulated fit (orange). For the simulation, acetyl-CoA labeling was assumed to follow a binomial distribution with a probability of 7.1% having m + 2 labeling form. i Validation of the method based on knock-down of IDH1 or IDH2 genes and following citrate isotopic labeling after feeding cells with [U-13C]-glutamine. Upon IDH1 knockdown, the ratios between citrate m + 5 and m + 4 in mitochondria and cytosol are similar, indicating that all reductive glutamine flux occurred in mitochondria (where citrate m + 4 is produced from malate m + 4). Meanwhile, IDH2

knockdown resulted in a higher citrate m + 5 to m + 4 ratio in cytosol, indicating that reductive IDH1 remains active. n.s. not significant. *P < 0.05 and **P < 0.01 by two-sample t-test. Data are mean ± SD, n = 3 independent biological replicates

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