abcam

ab246343

Anti-IDH2 antibody [EPR7576] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR7576
Purification technique	Affinity purification Protein A
Concentration	1.034 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
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	+4°C
Storage information	Do Not Freeze

Notes

ab246343 is the carrier-free version of ab129180.

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.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

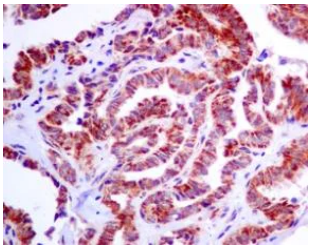
We do not recommend this combination. It is not covered by our product promise.

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

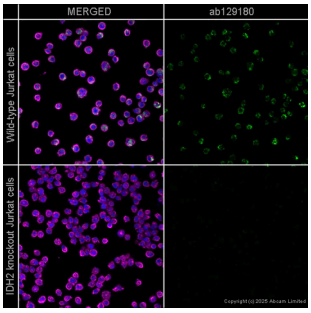


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

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

ab129180, at 1/250 dilution, staining IDH2 in Formalin-fixed, Paraffin-embedded Human thyroid gland carcinoma by Immunohistochemistry.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129180).

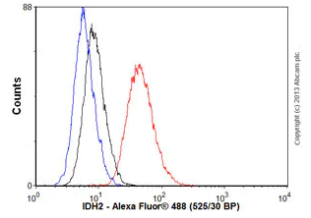
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

IDH2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-IDH2 antibody

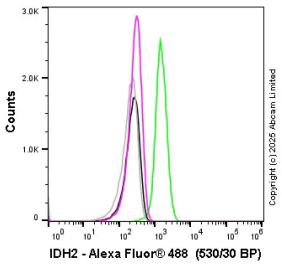
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat IDH2 knockout cells labeling IDH2 with ab129180 at 0.2 µg/ml. Cells were counterstained with ab7291 Anti-alpha Tubulin antibody [DM1A] at 1 : 1000 (1 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488 ab150081, green) and goat anti mouse IgG (Alexa Fluor® 594 ab150120, magenta) were used as the secondary antibodies at 1 : 1000 (2 µg/ml) dilution. The nuclear counterstain was DAPI (Blue).



Flow Cytometry (Intracellular) - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

IDH2 Flow Cytometry (Intracellular) staining using rabbit Anti-IDH2 antibody

Overlay histogram showing MCF7 cells stained with ab129180 (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 (ab129180, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. 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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129180).



Flow Cytometry (Intracellular) - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

IDH2 Flow Cytometry (Intracellular) staining of Jurkat using rabbit Anti-IDH2 antibody

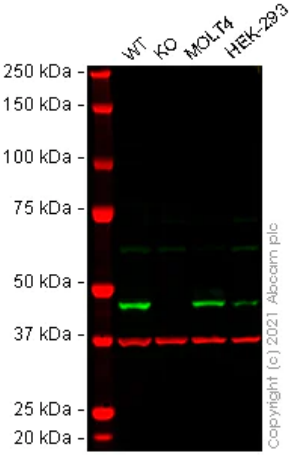
This data was developed using ab129180, the same antibody clone in a different buffer formulation.

Flow cytometry overlay histogram showing wild-type Jurkat (green line) and IDH2 knockout Jurkat stained with ab129180 (magenta line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab129180) (1x 10^6 in 100µl at 0.008 µg/ml (1/111625)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C.

Isotype control antibody was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control in Jurkat WT cells (black line) and Jurkat-IDH2 KO cells (grey line), used at the same concentration and conditions as the primary antibody.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

IDH2 Western blot staining using rabbit Anti-IDH2 antibody

False colour image of Western blot: Anti-IDH2 antibody [EPR7576] 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, ab129180 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-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-IDH2 antibody [EPR7576] (ab129180) 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)

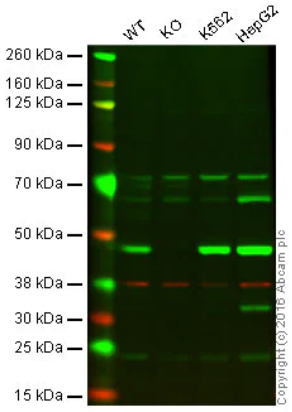
Lane 3:
MOLT-4 cell lysate at 20 µg

Lane 4:
HEK-293 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 48 kDa



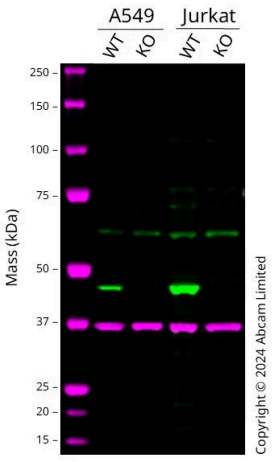
Western blot - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

IDH2 Western blot staining using rabbit Anti-IDH2 antibody

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: IDH2 knockout HAP1 cell lysate (20 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab129180 observed at 47 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab129180 was shown to recognize IDH2 when IDH2 knockout samples were used, along with additional cross-reactive bands. Wild-type and IDH2 knockout samples were subjected to SDS-PAGE. ab129180 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 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 h at room temperature before imaging.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129180).

All lanes:
Western blot - Anti-IDH2 antibody [EPR7576] (ab129180)

Predicted band size: 50 kDa



Western blot - Anti-IDH2 antibody [EPR7576] - BSA and Azide free (ab246343)

IDH2 Western blot staining using rabbit Anti-IDH2 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab129180).

Western blot: Anti-IDH2 antibody [EPR7576] (ab129180) 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, ab129180 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-IDH2 antibody [EPR7576] (ab129180) 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 51 kDa

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