abcam

Product datasheet

Anti-IDH2 antibody [EPR7576] ab129180

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-IDH2 antibody [EPR7576]
Description	Rabbit monoclonal [EPR7576] to IDH2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P **Unsuitable for:** ICC/IF or IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide within Human IDH2 aa 50-150. The exact sequence is proprietary.
Positive control	Human thyroid gland carcinoma tissue; Molt-4, K562, 293T and HepG2 whole cell lysate (**ab7900**).
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Tissue culture supernatant
Clonality	Monoclonal

Clone number	EPR7576
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab129180 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

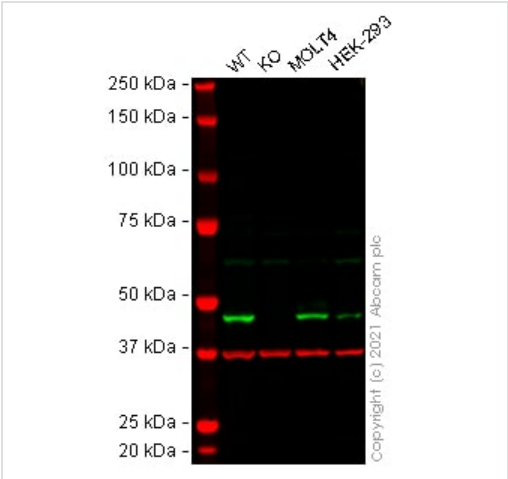
Application	Abreviews	Notes
Flow Cyt (Intra)		1/1000 - 1/10000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (3)	1/1000 - 1/10000. Detects a band of approximately 45 kDa (predicted molecular weight: 51 kDa).
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Application notes Is unsuitable for ICC/IF or IP.

Target

Function	Plays a role in intermediary metabolism and energy production. It may tightly associate or interact with the pyruvate dehydrogenase complex.
Involvement in disease	D-2-hydroxyglutaric aciduria 2 Glioma enetic variations are associated with cartilaginous tumors such as enchondroma or chondrosarcoma.
Sequence similarities	Belongs to the isocitrate and isopropylmalate dehydrogenases family.
Post-translational modifications	Acetylation at Lys-413 dramatically reduces catalytic activity. Deacetylated by SIRT3.
Cellular localization	Mitochondrion.

Images



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

All lanes : Anti-IDH2 antibody [EPR7576] (ab129180) at 1/1000 dilution

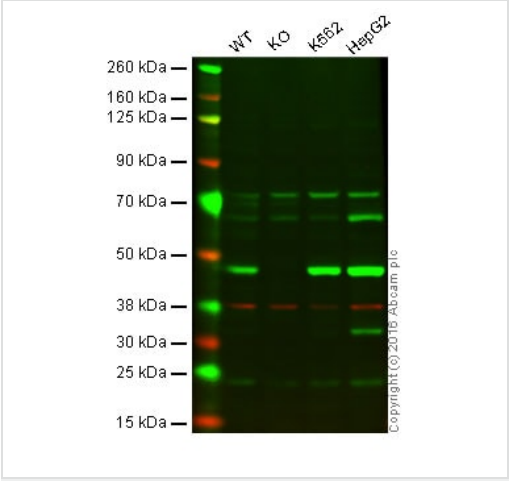
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : IDH2 knockout Jurkat cell lysate
Lane 3 : MOLT-4 cell lysate
Lane 4 : HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

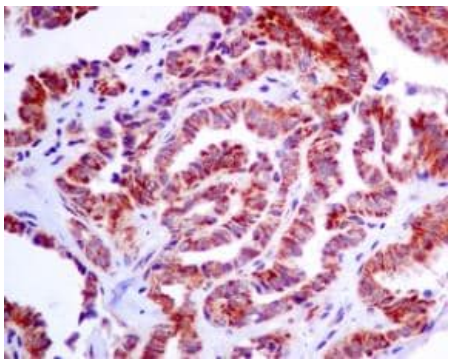
Predicted band size: 51 kDa
Observed band size: 48 kDa

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.



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

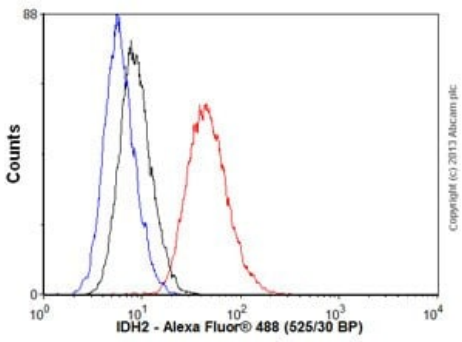
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: IDH2 knockout HAP1 cell lysate (20 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab129180 observed at 47 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab129180 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IDH2 antibody [EPR7576] (ab129180)

ab129180, at 1/250 dilution, staining IDH2 in Formalin-fixed, Paraffin-embedded Human thyroid gland carcinoma by Immunohistochemistry.

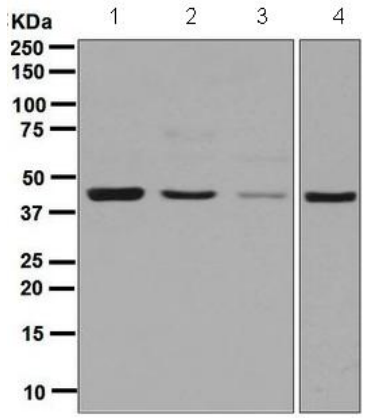
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-IDH2 antibody [EPR7576] (ab129180)

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.



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

All lanes : Anti-IDH2 antibody [EPR7576] (ab129180) at 1/1000 dilution

Lane 1 : Molt-4 cell lysate
Lane 2 : K562 cell lysate
Lane 3 : 293T cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-rabbit HRP

Predicted band size: 51 kDa
Observed band size: 45 kDa

Anti-IDH2 antibody [EPR7576] (ab129180)

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