abcam

Product datasheet

Anti-IDH2 antibody [EPR7577] - BSA and Azide free ab230796

KO VALIDATED Recombinant RabMAb

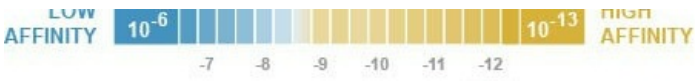
1 Abreviews 1 References 9 Images

Overview

Product name	Anti-IDH2 antibody [EPR7577] - BSA and Azide free
Description	Rabbit monoclonal [EPR7577] to IDH2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	MOLT4, K562, U87-M, and HepG2 cell lysates; Human liver tissue
General notes	ab230796 is the carrier-free version of **ab131263**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 4.70 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR7577
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab230796 in the following tested applications.

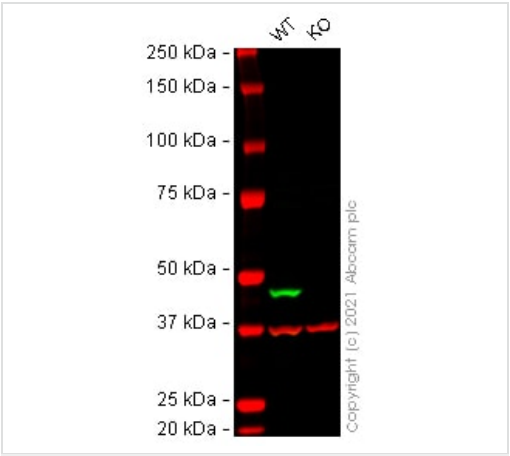
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 45 kDa (predicted molecular weight: 50 kDa).
IHC-P	★★★★☆ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

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 [EPR7577] - BSA and Azide free (ab230796)

All lanes : Anti-IDH2 antibody [EPR7577] (**ab131263**) at 1/1000 dilution

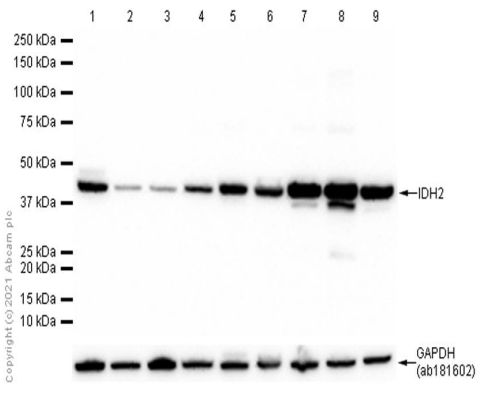
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : IDH2 knockout Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 50 kDa

False colour image of Western blot: Anti-IDH2 antibody [EPR7577] 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, **ab131263** 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 [EPR7577] - BSA and Azide free (ab230796)

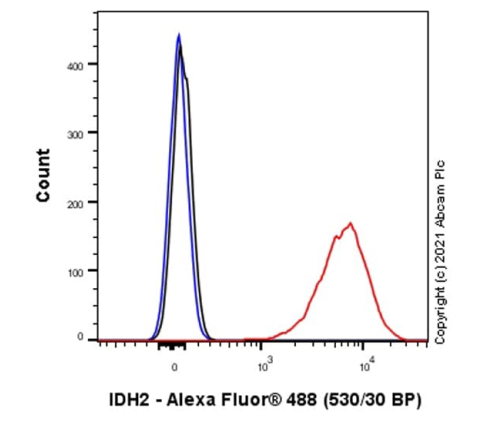
Lanes 1-7 : Anti-IDH2 antibody [EPR7577] (**ab131263**) at 1/5000 dilution (Purified)
Lanes 8-9 : Anti-IDH2 antibody [EPR7577] (**ab131263**) at 1/5000 dilution

Lane 1 : MOLT-4 (Human lymphoblastic leukemia T lymphoblast) whole cell lysate
Lane 2 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate
Lane 3 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 4 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 5 : Mouse liver lysate
Lane 6 : Rat liver lysate
Lane 7 : Mouse kidney lysate
Lane 8 : Rat kidney lysate
Lane 9 : Rat stomach lysate

Lysates/proteins at 20 µg per lane.

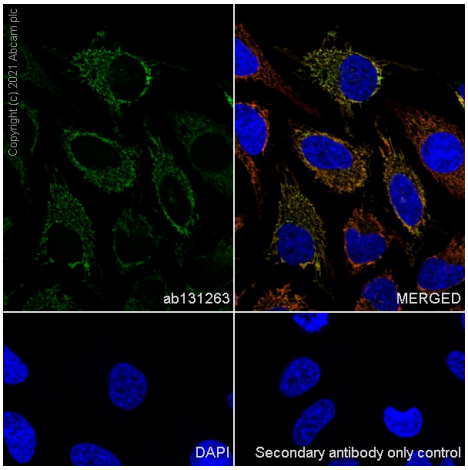
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 50 kDa



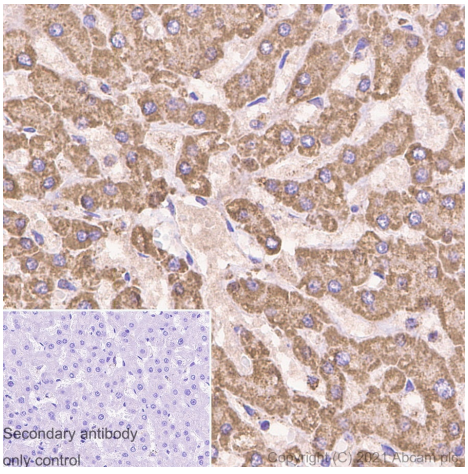
Flow Cytometry (Intracellular) - Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

This data was developed using ab230796, the same antibody clone in a different buffer formulation.
Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labelling IDH2 with Purified ab230796 at 1:20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150081**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



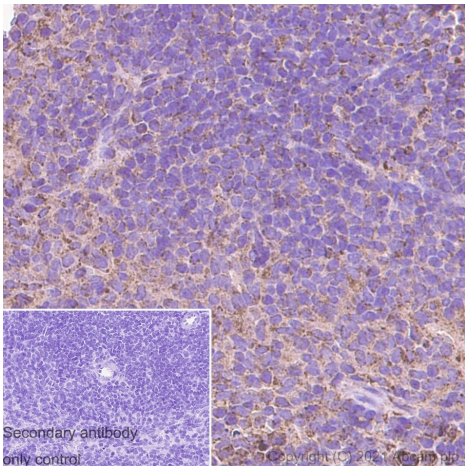
Immunocytochemistry/ Immunofluorescence - Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

This data was developed using ab230796, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling IDH2 with Purified ab230796 at 1:1000 dilution (0.2 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



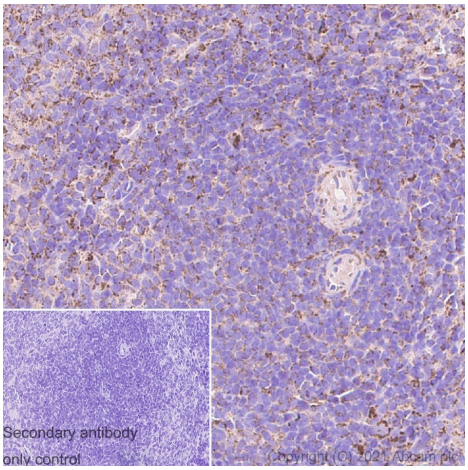
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

This data was developed using **ab131263**, the same antibody clone in a different buffer formulation.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue sections labeling IDH2 with Purified **ab131263** at 1:100 (2.11 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



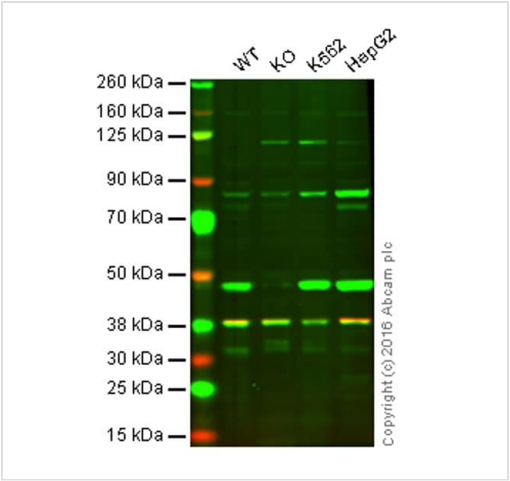
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

This data was developed using **ab131263**, the same antibody clone in a different buffer formulation. Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue sections labeling IDH2 with Purified **ab131263** at 1:100 (2.11 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

This data was developed using **ab131263**, the same antibody clone in a different buffer formulation. Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue sections labeling IDH2 with Purified **ab131263** at 1:100 (2.11 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

This WB data was generated using the same anti-IDH2 antibody clone, EPR7577, in a different buffer formulation (cat# **ab131263**).

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

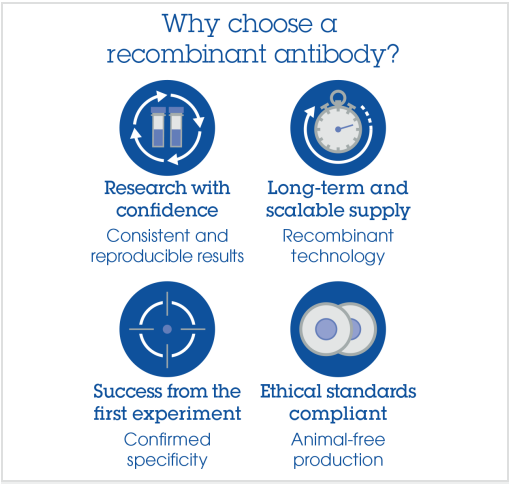
Lane 2: IDH2 knockout HAP1 cell lysate (20 µg)

Lane 3: K562 cell lysate (20 µg)

Lane 4: HepG2 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - **ab131263** observed at 48 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab131263 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. **ab131263** and **ab8245** (loading control to GAPDH) were both diluted 1/10 000 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.



Anti-IDH2 antibody [EPR7577] - BSA and Azide free (ab230796)

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