abcam

Product datasheet

Anti-IDH1 antibody [EPR21002] - BSA and Azide free ab242078



Recombinant

RabMAb

10 Images

Overview

Product name Anti-IDH1 antibody [EPR21002] - BSA and Azide free

Description Rabbit monoclonal [EPR21002] to IDH1 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, IP

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: SH-SY5Y, HAP1 and HeLa cell lysates. IHC-P: Human stomach, glioblastoma and

endometrium cancer tissues; Mouse and rat kidney tissues. Flow Cyt (intra): HeLa cells. IP: SH-

SY5Y whole cell lysate.

General notes ab242078 is the carrier-free version of <u>ab230949</u>.

Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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.

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

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monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR21002

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab242078 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.
WB		Use at an assay dependent concentration. Detects a band of approximately 47 kDa (predicted molecular weight: 46 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

Target

Involvement in disease Glioma

Genetic variations are associated with cartilaginous tumors such as enchondroma or

chondrosarcoma. Mutations of Arg-132 to Cys, Gly or His abolish the conversion of isocitrate to

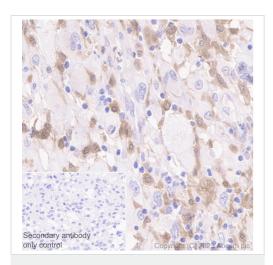
alpha-ketoglutarate. Instead, alpha-ketoglutarate is converted to R(-)-2-hydroxyglutarate.

Sequence similaritiesBelongs to the isocitrate and isopropylmalate dehydrogenases family.

Post-translational modifications

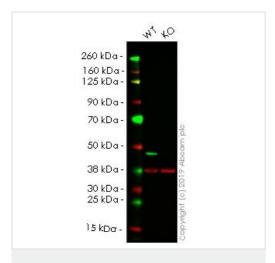
Acetylation at Lys-374 dramatically reduces catalytic activity.

Cellular localization Cytoplasm. Peroxisome.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IDH1 antibody

[EPR21002] - BSA and Azide free (ab242078)



Western blot - Anti-IDH1 antibody [EPR21002] - BSA and Azide free (ab242078)

Immunohistochemical analysis of paraffin-embedded human glioblastoma tissue labeling IDH1 with <u>ab230949</u> at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP). Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Positive staining on human glioblastoma. The section was incubated with <u>ab230949</u> at 4°C overnight.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230949).

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All lanes : Anti-IDH1 antibody [EPR21002] (**ab230949**) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: IDH1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 46 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab230949</u>).

Lanes 1-2: Merged signal (red and green). Green - <u>ab230949</u> observed at 46 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

<u>ab230949</u> was shown to react with IDH1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line <u>ab264916</u> (knockout cell lysate <u>ab257221</u>) was used. Wild-type HeLa and IDH1 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. <u>ab230949</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®]800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®]680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Secondary antibody only control

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IDH1 antibody

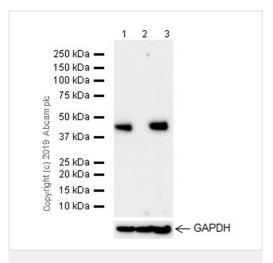
[EPR21002] - BSA and Azide free (ab242078)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling IDH1 with <u>ab230949</u> at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic and nuclear staining in rat kidney (PMID:30153799). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230949).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-IDH1 antibody [EPR21002] - BSA and Azide free (ab242078)

All lanes: Anti-IDH1 antibody [EPR21002] (ab230949) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: IDH1 knockout HAP1 whole cell lysate

Lane 3 : SH-SY5Y (human neuroblastoma epithelial cell), whole cell lysate

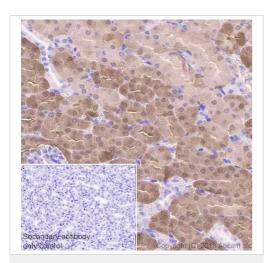
Lysates/proteins at 10 µg per lane.

Predicted band size: 46 kDa

Exposure time: 62 seconds

<u>ab230949</u> was shown to specifically react with IDH1 in wild-type HAP1 cells as signal was lost in IDH1 knockout cells. Wild-type and IDH1 knockout samples were subjected to SDS-PAGE. <u>ab230949</u> and <u>ab181602</u> (Rabbit anti-GAPDH loading control) were

incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IDH1 antibody

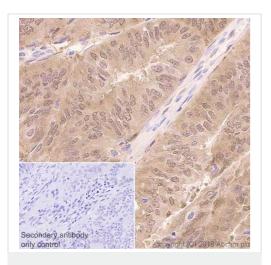
[EPR21002] - BSA and Azide free (ab242078)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling IDH1 with **ab230949** at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic and nuclear staining in mouse kidney (PMID:30153799). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230949).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IDH1 antibody

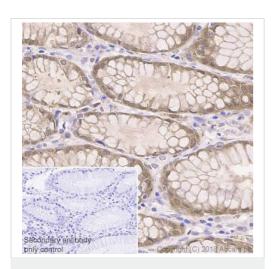
[EPR21002] - BSA and Azide free (ab242078)

Immunohistochemical analysis of paraffin-embedded human endometrium cancer tissue labeling IDH1 with <u>ab230949</u> at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic and nuclear staining in human endometrium cancer (PMID:29921847). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230949).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IDH1 antibody

[EPR21002] - BSA and Azide free (ab242078)

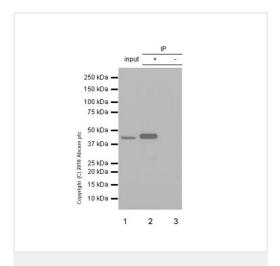
Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling IDH1 with <u>ab230949</u> at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic and nucleus staining in human stomach (PMID:27466503). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230949).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-IDH1 antibody
[EPR21002] - BSA and Azide free (ab242078)

IDH1 was immunoprecipitated from 0.35 mg of SH-SY5Y (human neuroblastoma cell line from bone marrow) whole cell lysate with **ab230949** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab230949** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: SH-SY5Y whole cell lysate 10 µg (Input).

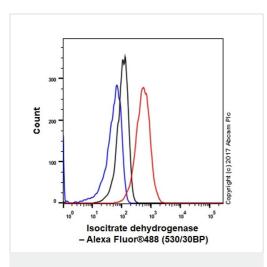
Lane 2: ab230949 IP in SH-SY5Y whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab230949</u> in SH-SY5Y whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab230949</u>).



Flow Cytometry (Intracellular) - Anti-IDH1 antibody [EPR21002] - BSA and Azide free (ab242078)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling IDH1 with <u>ab230949</u> at 1/60 dilution (**red**) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (**black**) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (**blue**). Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230949).



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