

Anti-IDH1 Antibody Picoband™ (monoclonal, 16H7)

Catalog # ABO14927

Product Information

Application	WB, IF, ICC, FC
Primary Accession	O75874
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Liquid
Description	Anti-IDH1 Antibody Picoband™ (monoclonal, 16H7) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Additional Information

Gene ID	3417
Other Names	Isocitrate dehydrogenase [NADP] cytoplasmic, IDH, IDH1, 1.1.1.42, Cytosolic NADP-isocitrate dehydrogenase, IDPc, NADP(+)-specific ICDH, Oxalosuccinate decarboxylase, IDH1, PICD
Calculated MW	46659
Application Details	Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Subcellular Localization	Cytoplasm. Peroxisome.
Source	Eukaryota
Protein Name	Isocitrate dehydrogenase [NADP] cytoplasmic
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 16H7
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human IDH1, different from the related mouse and rat sequences by one amino acid.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20 °C for one year from date of receipt. After reconstitution, at 4 °C for one month. It can also be aliquotted and stored frozen at -20 °C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	IDH1
Synonyms	PICD
Function	Catalyzes the NADP(+)-dependent oxidative decarboxylation of isocitrate (D-threo-isocitrate) to 2-ketoglutarate (2-oxoglutarate), which is required by other enzymes such as the phytanoyl-CoA dioxygenase (PubMed: 10521434 , PubMed: 19935646). Plays a critical role in the generation of NADPH, an important cofactor in many biosynthesis pathways (PubMed: 10521434). May act as a corneal epithelial crystallin and may be involved in maintaining corneal epithelial transparency (By similarity).
Cellular Location	Cytoplasm, cytosol. Peroxisome

Background

Isocitrate dehydrogenase 1 (NADP+), soluble is an enzyme that in humans is encoded by the IDH1 gene. Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. These enzymes belong to two distinct subclasses, one of which utilizes NAD (+) as the electron acceptor and the other NADP (+). Five isocitrate dehydrogenases have been reported: three NAD (+)-dependent isocitrate dehydrogenases, which localize to the mitochondrial matrix, and two NADP (+)-dependent isocitrate dehydrogenases, one of which is mitochondrial and the other predominantly cytosolic. Each NADP (+)-dependent isozyme is a homodimer. The protein encoded by this gene is the NADP (+)-dependent isocitrate dehydrogenase found in the cytoplasm and peroxisomes. It contains the PTS-1 peroxisomal targeting signal sequence. The presence of this enzyme in peroxisomes suggests roles in the regeneration of NADPH for intraperoxisomal reductions, such as the conversion of 2, 4-dienoyl-CoAs to 3-enoyl-CoAs, as well as in peroxisomal reactions that consume 2-oxoglutarate, namely the alpha-hydroxylation of phytanic acid. The cytoplasmic enzyme serves a significant role in cytoplasmic NADPH production. Alternatively spliced transcript variants encoding the same protein have been found for this gene.

Images

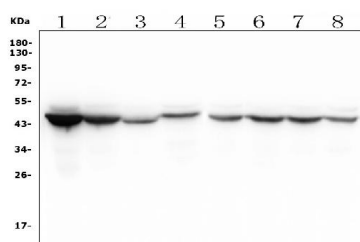


Figure 1. Western blot analysis of IDH1 using anti-IDH1 antibody (M00129-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human HepG2 tissue lysates,
Lane 2: human Caco-2 whole cell lysates,
Lane 3: human U-87MG whole cell lysates,
Lane 4: human THP-1 whole cell lysates,
Lane 5: human Hela whole cell lysates.
Lane 6: human K562 whole cell lysates.
Lane 7: human PC-3 whole cell lysates.
Lane 8: human HEK293 whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-IDH1 antigen affinity purified polyclonal antibody (Catalog # M00129-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each

and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for IDH1 at approximately 47KD. The expected band size for IDH1 is at 47KD.

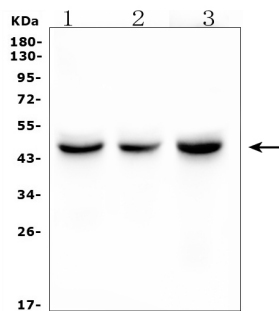


Figure 2. Western blot analysis of IDH1 using anti-IDH1 antibody (M00129-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: rat liver tissue lysates,

Lane 2: rat RH35 whole cell lysates,

Lane 3: mouse liver whole cell lysates,

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-IDH1 antigen affinity purified polyclonal antibody (Catalog # M00129-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for IDH1 at approximately 47KD. The expected band size for IDH1 is at 47KD.

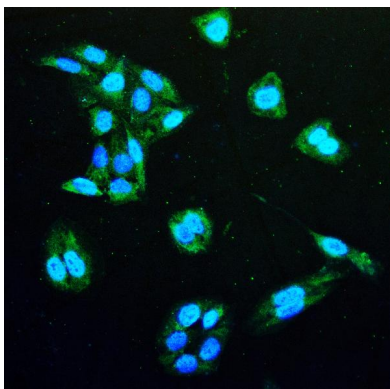


Figure 3. IF analysis of IDH1 using anti-IDH1 antibody (M00129-1).

IDH1 was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-IDH1 Antibody (M00129-1) overnight at 4°C. DyLight488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

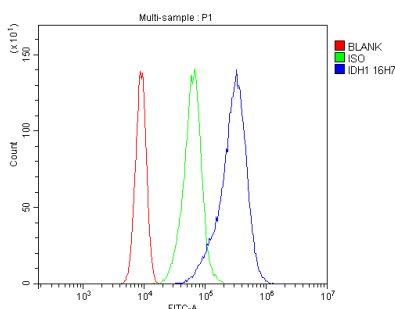


Figure 4. Flow Cytometry analysis of CACO-2 cells using anti-IDH1 antibody (M00129-1).

Overlay histogram showing CACO-2 cells stained with M00129-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IDH1 Antibody (M00129-1, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight@488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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