

Anti-Aldolase/ALDOA Antibody Picoband™ (monoclonal, 6H8)

Catalog # ABO14982

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P04075
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Aldolase/ALDOA Antibody Picoband™ (monoclonal, 6H8) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	226
Other Names	Fructose-bisphosphate aldolase A, 4.1.2.13, Lung cancer antigen NY-LU-1, Muscle-type aldolase, ALDOA (HGNC:414), ALDA
Calculated MW	39420
Application Details	Western blot, 0.25-0.5 µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry, 1- 10 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 6H8
Immunogen	E.coli-derived human Aldolase/ALDOA recombinant protein (Position: E50-Y364).
Purification	Immunogen affinity purified.
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	ALDOA (HGNC:414)
Synonyms	ALDA
Function	Catalyzes the reversible conversion of beta-D-fructose 1,6- biphosphate (FBP) into two triose phosphate and plays a key role in glycolysis and gluconeogenesis (PubMed: 14766013). In addition, also functions as a scaffolding protein (By similarity). In response to glucose deprivation, FBP dissociates from aldolase and acts as an adapter that promotes AMP-activated protein kinase (AMPK) activity: mechanistically, associates with transient receptor potential channels TrpV (TRPV1-TRPV4), promoting inhibition of the V-ATPase complex on lysosomes and AMPK activation via the AXIN1-STK11/LKB1 axis (By similarity).
Cellular Location	Cytoplasm, myofibril, sarcomere, I band {ECO:0000250 UniProtKB:P00883}. Cytoplasm, myofibril, sarcomere, M line {ECO:0000250 UniProtKB:P00883}. Note=In skeletal muscle, accumulates around the M line and within the I band, colocalizing with FBP2 on both sides of the Z line in the absence of Ca(2+) {ECO:0000250 UniProtKB:P00883}

Background

Aldolase A (ALDOA, or ALDA), also known as fructose-bisphosphate aldolase, is an enzyme that in humans is encoded by the ALDOA gene on chromosome 16. This gene encodes a member of the class I fructose-bisphosphate aldolase protein family. The encoded protein is a glycolytic enzyme that catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Three aldolase isozymes (A, B, and C), encoded by three different genes, are differentially expressed during development. Mutations in this gene have been associated with Glycogen Storage Disease XII, an autosomal recessive disorder associated with hemolytic anemia. Disruption of this gene also plays a role in the progression of multiple types of cancers. Related pseudogenes have been identified on chromosomes 3 and 10.

Images

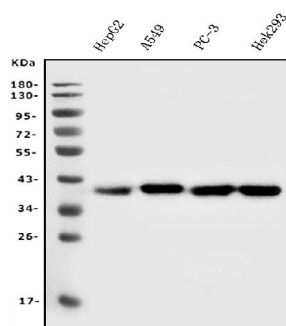


Figure 1. Western blot analysis of Aldolase/ALDOA using anti-Aldolase/ALDOA antibody (M05022-2).

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 whole cell lysates,
Lane 2: human A549 whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: 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-Aldolase/ALDOA antigen affinity purified monoclonal antibody (Catalog # M05022-2) 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 Aldolase/ALDOA at approximately 39KD. The expected band size for Aldolase/ALDOA is at 39KD.

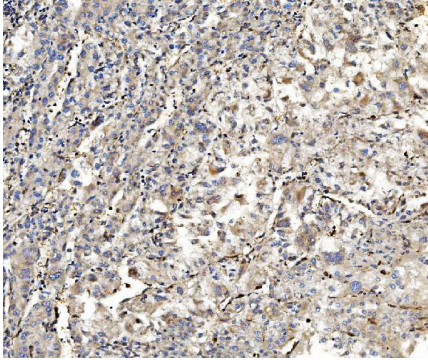


Figure 2. IHC analysis of Aldolase/ALDOA using anti-Aldolase/ALDOA antibody (M05022-2). Aldolase/ALDOA was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Aldolase/ALDOA Antibody (M05022-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

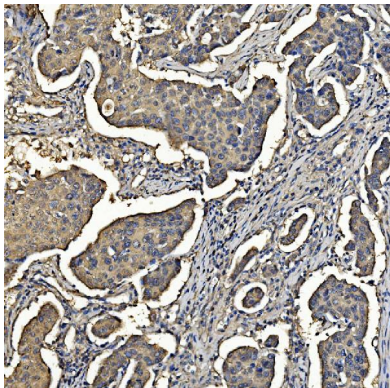


Figure 3. IHC analysis of Aldolase/ALDOA using anti-Aldolase/ALDOA antibody (M05022-2). Aldolase/ALDOA was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Aldolase/ALDOA Antibody (M05022-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

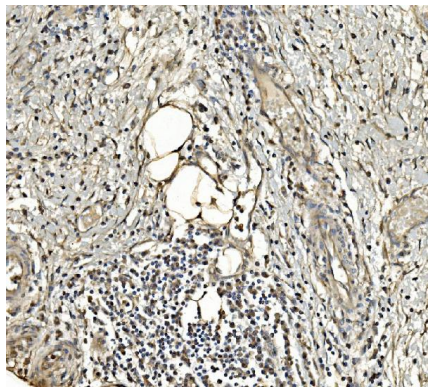


Figure 4. IHC analysis of Aldolase/ALDOA using anti-Aldolase/ALDOA antibody (M05022-2). Aldolase/ALDOA was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Aldolase/ALDOA Antibody (M05022-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

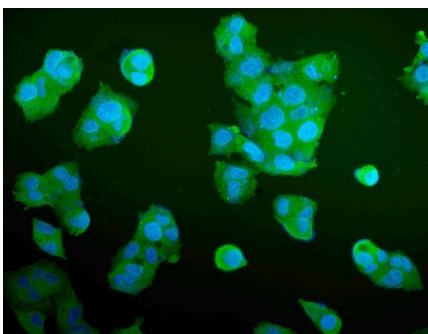


Figure 5. IF analysis of Aldolase/ALDOA using anti-Aldolase/ALDOA antibody (M05022-2). Aldolase/ALDOA was detected in immunocytochemical section of HEPG2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Aldolase/ALDOA Antibody (M05022-2) overnight at 4°C. DyLight®488 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.

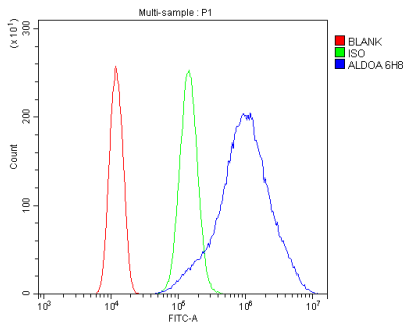


Figure 6. Flow Cytometry analysis of SiHa cells using anti-Aldolase/ALDOA antibody (M05022-2). Overlay histogram showing SiHa cells stained with M05022-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Aldolase/ALDOA Antibody (M05022-2, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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