

Anti-DHODH Antibody Picoband™ (monoclonal, 2G7)

Catalog # ABO15107

Product Information

Application	WB, IHC
Primary Accession	Q02127
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-DHODH Antibody Picoband™ (monoclonal, 2G7) . Tested in IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	1723
Other Names	Dihydroorotate dehydrogenase (quinone), mitochondrial, DHodehase, 1.3.5.2, Dihydroorotate oxidase, DHODH
Calculated MW	42867
Application Details	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 2G7
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human DHODH, different from the related mouse sequence by four amino acids, and from the related rat sequence by two amino acids.
Purification	Immunogen affinity purified.
Storage	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 freezing and thawing.

Protein Information

Name	DHODH
-------------	-------

Function

Catalyzes the conversion of dihydroorotate to orotate with quinone as electron acceptor. Required for UMP biosynthesis via de novo pathway.

Cellular Location

Mitochondrion inner membrane; Single-pass membrane protein

Background

Dihydroorotate dehydrogenase (DHODH) is an enzyme that in humans is encoded by the DHODH gene on chromosome 16. The protein encoded by this gene catalyzes the fourth enzymatic step, the ubiquinone-mediated oxidation of dihydroorotate to orotate, in de novo pyrimidine biosynthesis. This protein is a mitochondrial protein located on the outer surface of the inner mitochondrial membrane.

Images

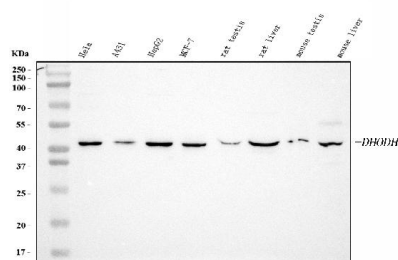


Figure 1. Western blot analysis of DHODH using anti-DHODH antibody (M04035-1).

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 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,
Lane 2: human A431 whole cell lysates,
Lane 3: human HepG2 whole cell lysates,
Lane 4: human MCF-7 whole cell lysates,
Lane 5: rat testis tissue lysates,
Lane 6: rat liver tissue lysates,
Lane 7: mouse testis tissue lysates,
Lane 8: mouse liver tissue lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-DHODH antigen affinity purified monoclonal antibody (Catalog # M04035-1) 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 DHODH at approximately 43 kDa. The expected band size for DHODH is at 43 kDa.

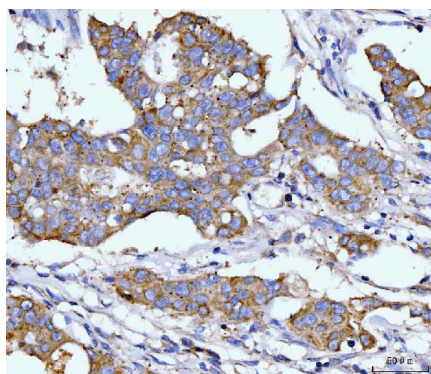


Figure 2. IHC analysis of DHODH using anti-DHODH antibody (M04035-1).

DHODH was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-DHODH Antibody (M04035-1) 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.

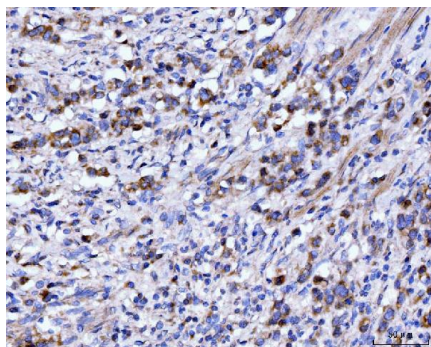


Figure 3. IHC analysis of DHODH using anti-DHODH antibody (M04035-1). DHODH was detected in a paraffin-embedded section of human gastric cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml mouse anti-DHODH Antibody (M04035-1) 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.

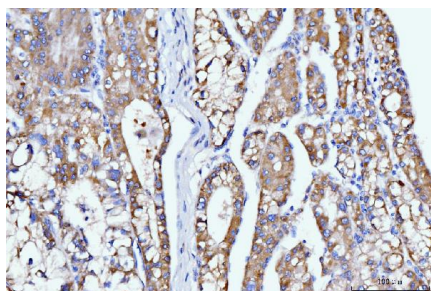


Figure 4. IHC analysis of DHODH using anti-DHODH antibody (M04035-1). DHODH was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml mouse anti-DHODH Antibody (M04035-1) 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.

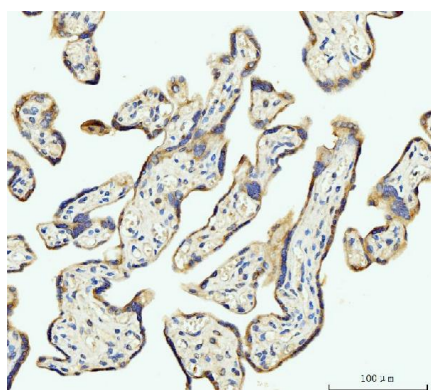


Figure 5. IHC analysis of DHODH using anti-DHODH antibody (M04035-1). DHODH was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml mouse anti-DHODH Antibody (M04035-1) 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.

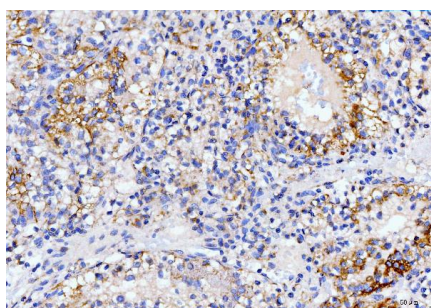


Figure 6. IHC analysis of DHODH using anti-DHODH antibody (M04035-1). DHODH was detected in a paraffin-embedded section of human renal carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml mouse anti-DHODH Antibody (M04035-1) 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.