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Anti-FH Antibody Picoband™ (monoclonal, 9D8)

Catalog # ABO14823

Product Information

Application	WB, IHC, IF, ICC
Primary Accession	P07954
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-FH Antibody Picoband™ (monoclonal, 9D8) . Tested in IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.

Additional Information

Gene ID	2271
Other Names	Fumarate hydratase, mitochondrial, Fumarase {ECO:0000303|PubMed:27037871, ECO:0000303|PubMed:3828494, ECO:0000303|Ref.2}, HsFH, 4.2.1.2, FH {ECO:0000303|PubMed:27037871, ECO:0000312|HGNC:HGNC:3700}
Calculated MW	54637
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 5 □g/ml
Subcellular Localization	Isoform Mitochondrial: Mitochondrion.
Tissue Specificity	Expressed in red blood cells; underexpressed in red blood cells (cytoplasm) of patients with hereditary non- spherocytic hemolytic anemia of unknown etiology.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 9D8
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human FH, which shares 100% and 97.8% amino acid (aa) sequence identity with mouse and rat FH, respectively.
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	FH {ECO:0000303|PubMed:27037871, ECO:0000312|HGNC:HGNC:3700}
Function	Catalyzes the reversible stereospecific interconversion of fumarate to L-malate (PubMed:30761759). Experiments in other species have demonstrated that specific isoforms of this protein act in defined pathways and favor one direction over the other (Probable).
Cellular Location	[Isoform Mitochondrial]: Mitochondrion
Tissue Location	Expressed in red blood cells; underexpressed in red blood cells (cytoplasm) of patients with hereditary non-spherocytic hemolytic anemia of unknown etiology.

Background

Fumarase (or fumaratehydratase) is an enzyme that catalyzes the reversible hydration/dehydration of fumarate to malate. Fumarase comes in two forms: mitochondrial and cytosolic. The mitochondrial isoenzyme is involved in the Krebs Cycle (also known as the Tricarboxylic Acid Cycle [TCA] or the Citric Acid Cycle), and the cytosolic isoenzyme is involved in the metabolism of amino acids and fumarate. Subcellular localization is established by the presence of a signal sequence on the amino terminus in the mitochondrial form, while subcellular localization in the cytosolic form is established by the absence of the signal sequence found in the mitochondrial variety. This enzyme participates in 2 metabolic pathways: citric acid cycle, reductive citric acid cycle (CO2 fixation), and is also important in renal cell carcinoma. Mutations in this gene have been associated with the development of leiomyomas in the skin and uterus in combination with renal cell carcinoma.

Images

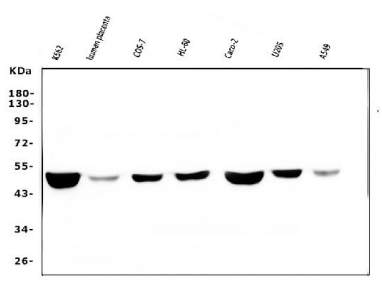


Figure 1. Western blot analysis of FH using anti-FH antibody (M02097).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: K562 whole cell lysates,
Lane 2: human placenta tissue lysates,
Lane 3: COS-7 whole cell lysates,
Lane 4: HL-60 whole cell lysates,
Lane 5: Caco-2 whole cell lysates,
Lane 6: U20S whole cell lysates,
Lane 7: A549 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-FH antigen affinity purified monoclonal antibody (Catalog # M02097) 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 1:5000 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 FH at approximately 48KD. The expected band size for FH is at 48KD.

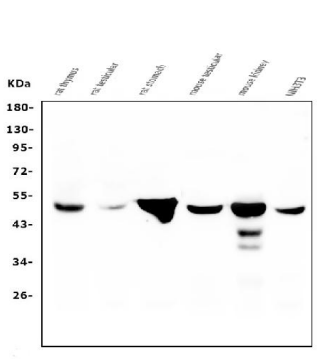


Figure 2. Western blot analysis of FH using anti-FH antibody (M02097).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat thymus tissue lysates,
Lane 2: rat testicular tissue lysates,
Lane 3: rat stomach tissue lysates,
Lane 4: mouse testicular tissue lysates,
Lane 5: mouse kidney tissue lysates,
Lane 6: NIH3T3 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-FH antigen affinity purified monoclonal antibody (Catalog # M02097) 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 1:5000 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 FH at approximately 48KD. The expected band size for FH is at 48KD.

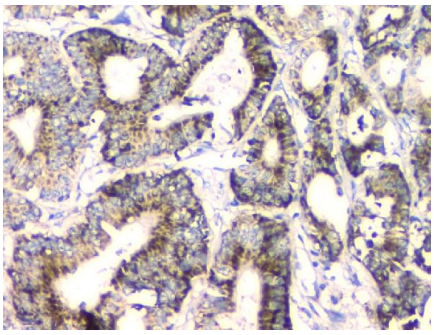


Figure 3. IHC analysis of FH using anti-FH antibody (M02097).
FH was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-FH Antibody (M02097) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

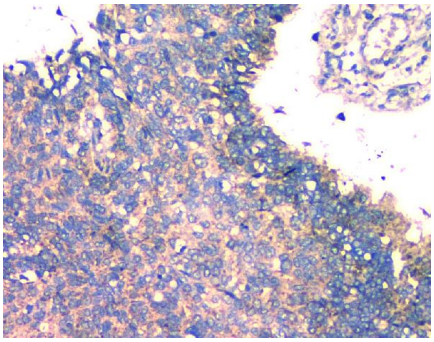


Figure 4. IHC analysis of FH using anti-FH antibody (M02097).
FH was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-FH Antibody (M02097) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

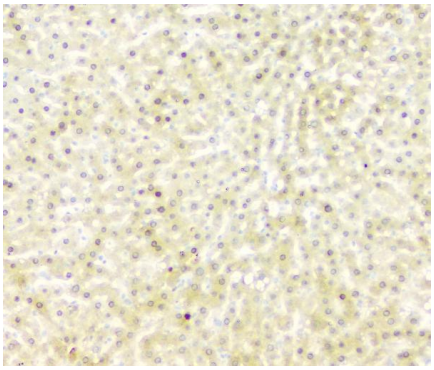


Figure 5. IHC analysis of FH using anti-FH antibody (M02097).
FH was detected in paraffin-embedded section of rat liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-FH Antibody (M02097) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.