10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

# Anti-DHODH Antibody Picoband™ (monoclonal, 4E3)

Catalog # ABO15082

## Product Information

| | |
|---|---|
| **Application** | WB, IF, ICC, FC |
| **Primary Accession** | Q02127 |
| **Host** | Mouse |
| **Isotype** | Mouse IgG2b |
| **Reactivity** | Rat, Human, Mouse |
| **Clonality** | Monoclonal |
| **Format** | Lyophilized |
| **Description** | Anti-DHODH Antibody Picoband™ (monoclonal, 4E3) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. |
| **Reconstitution** | Adding 0.2 ml of distilled water will yield a concentration of 500 □g/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 □g/ml, Human, Mouse, Rat<br>Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human<br>Flow Cytometry, 1-3 □g/1x10^6 cells, Human |
| **Source** | Eukaryota |
| **Contents** | Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4. |
| **Clone Names** | Clone: 4E3 |
| **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

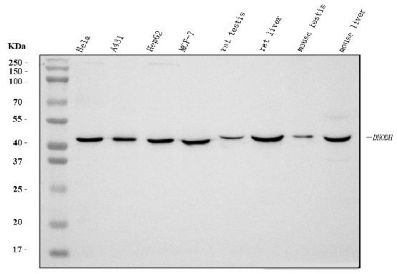


Figure 1. Western blot analysis of DHODH using anti-DHODH antibody (M04035).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole 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) 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.

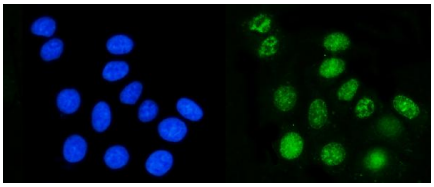

Figure 2. IF analysis of DHODH using anti-DHODH antibody (M04035).
DHODH was detected in an immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-DHODH Antibody (M04035) 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.

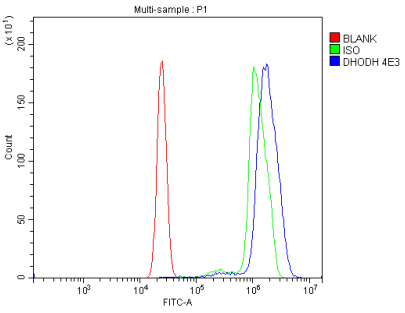


Figure 3. Flow Cytometry analysis of U937 cells using anti-DHODH antibody (M04035).
Overlay histogram showing U937 cells stained with M04035 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-DHODH Antibody (M04035, 1 µg/1x$10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.