abcam

Product datasheet

Malate Dehydrogenase 2 (MDH2) Activity Assay ab119693

9 References 4 Images

Overview

Product name Malate Dehydrogenase 2 (MDH2) Activity Assay

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
1	3			4.1%

Inter-assay

Sample	n	Mean	SD	CV%
1	3			13.9%

Sample type Cell culture extracts, Tissue Extracts

Assay type Enzyme activity

Sensitivity 0.78 µg/ml

Range 0.78 µg/ml - 200 µg/ml

Species reactivity **Reacts with:** Mouse, Rat, Human

Product overview Malate Dehydrogenase 2 (MDH2) Activity Assay (ab119693) is used to determine mitochondrial malate dehydrogenase activity (MDH2) in a sample. The enzyme is captured within the wells of the microplate and activity is determined by following the production of NADH in the following MDH2 catalyzed reaction: malate + NAD → oxaloacetic acid + NADH (↑ Absorbance at 450 nm). The generation of NADH is coupled to the 1:1 reduction of a reporter dye to yield a colored (yellow) reaction product whose concentration can be monitored by measuring the increase in absorbance at 450 nm. In each well, ab119693 immunocaptures only native MDH2 from the chosen sample; this removes all other enzymes, including MDH1 in cytosol.

This product allows researchers to focus on TCA cycle, studying isotype-specific malate dehydrogenase (MDH2) activity assay without the necessity of isolating mitochondria.

Notes Mitochondrial malate dehydrogenase (MDH2, P40926) is a 35.5 kDa enzyme that catalyzes the conversion of malate into oxaloacetate (using NAD+) and vice versa. (EC 1.1.1.37) Several isozymes of malate dehydrogenase exist, depending on where they are localized in the cell and

their specific dependence on NAD+ or NADP+ (only in chloroplasts). There are two main isoforms in eukaryotic cells. One is found in the mitochondrial matrix (MDH2), participating as a key enzyme in the citric acid cycle that catalyzes the oxidation of malate. The other is found in the cytoplasm (MDH1), assisting the malate-aspartate shuttle with exchanging reducing equivalents so that malate can pass through the mitochondrial membrane to be transformed into oxaloacetate for further cellular processes. Because malate dehydrogenase is closely tied to the citric acid cycle, regulation is highly dependent on TCA products. High malate concentrations stimulate MDH activity, and, in a converse manner, high oxaloacetate concentrations inhibit the enzyme. Enzyme activity is enhanced by acetylation.

Storage: All components are shipped cold. Reagent dye, coupler, malate and NAD+ are shipped lyophilized. Before use rehydrate by adding 0.25 mL pure H_2O to each tube and vortex each tube thoroughly to dissolve. After hydration unused amounts of these four materials should be stored at -80°C for 6 months. Store all other components at 4°C. This kit is stable for 6 months from receipt.

Platform Microplate reader

Properties

Storage instructions Store at +4°C. Please refer to protocols.

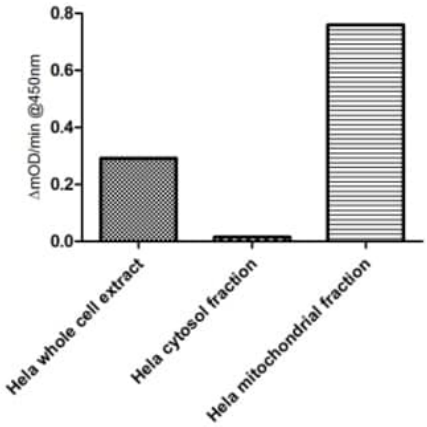
Components	1 x 96 tests
100X Coupler	1 unit
100X NAD+	1 unit
100X Reagent Dye	1 unit
100X Sodium Malate	1 unit
10X Blocking Buffer	1 x 8ml
20X Buffer	1 x 20ml
Base Buffer	1 x 24ml
Extraction Buffer (ab260490)	1 x 15ml
MDH2 Microplate	1 unit

Sequence similarities Belongs to the LDH/MDH superfamily. MDH type 1 family.

Post-translational modifications Acetylation is enhanced by up to 67% after treatment either with trichostin A (TSA) or with nicotinamide (NAM) with the appearance of tri-and tetraacetylations. Glucose also increases acetylation by about 60%.

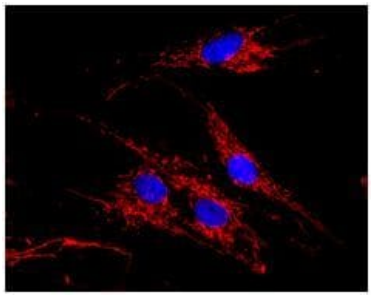
Cellular localization Mitochondrion matrix.

Images



ELISA - MDH2 Human Activity Assay Kit (ab119693)

Figures 7. The isoform specificity of the malate activity measured by this kit is confirmed by measuring the MDH activity from different cell fractions. Activity was only detected from the mitochondrial fraction (MDH2), not the cytosol fraction (MDH1).



Immunocytochemistry/ Immunofluorescence - MDH2 Human Activity Assay Kit (ab119693)

Figure 4. MDH2 antibody showing reactivity in a mitochondrial intracellular pattern with immunofluorescence microscopy.

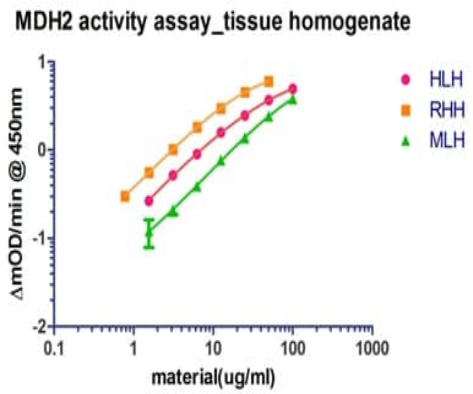


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Figure 2. MDH2 activity measurements of serially diluted human liver homogenate, rat heart homogenate, and mouse liver homogenate.

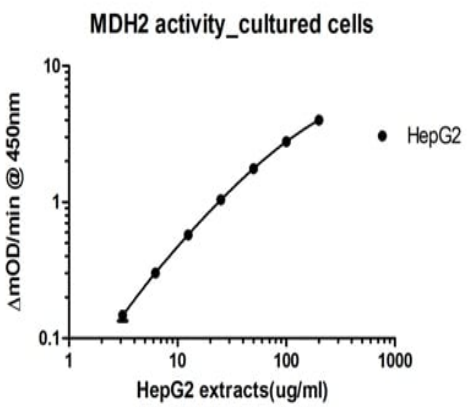

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Figure 1. MDH2 activity measurements of serially diluted cultured HepG2 cell extracts.

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