Recombinant Human MDH1 His-tag

Catalog Number: 11632-MH

DESCRIPTION

Source	*E. coli*-derived human MDH1 protein Met1-Ala334, with a C-terminal 6-His tag Accession # P40925.4
N-terminal Sequence Analysis	Met1 & Ser2
Predicted Molecular Mass	37 kDa

SPECIFICATIONS

SDS-PAGE	35-38 kDa, under reducing conditions
Activity	Measured by its ability to produce malate from oxaloacetate. The specific activity is >55,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 100 mM NaCl, pH 9.0
- Recombinant Human Malate Dehydrogenase 1 (rhMDH1) (Catalog # 11632-MH)
- β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
- Oxaloacetic Acid, 100 mM Stock in deionized water
- Clear 96-Well Plate
- Plate Reader with Absorbance Read Capability

Assay

1. Dilute rhMDH1 to 0.5 µg/mL in Assay Buffer.
2. Prepare a substrate mixture containing 1.6 mM NADH and 2 mM Oxaloacetic Acid in Assay Buffer.
3. In a plate, load 50 µL of 0.5 µg/mL rhMDH1 and start the reaction by adding 50 µL of substrate mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of substrate mixture.
4. Read plate at 340 nm (absorbance) in kinetic mode for 10 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol x (-1)}}{\varepsilon\text{** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$

***Using the path correction of 0.320 cm

Final Assay Conditions

Per Well:

- rhMDH1: 0.025 µg
- NADH: 0.8 mM
- Oxaloacetic Acid: 1 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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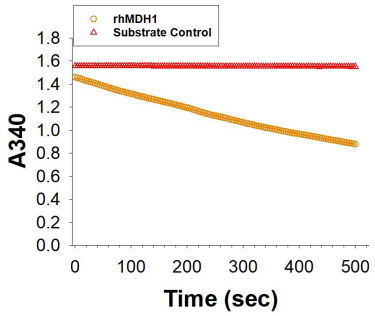
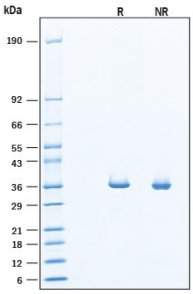
Enzyme Activity



Recombinant Human MDH1 His-tag Enzyme Activity. Recombinant Human MDH1 His-tag (Catalog # 11632-MH) is measured by its ability to produce malate from oxaloacetate.

SDS-PAGE



Recombinant Human MDH1 His-tag SDS-PAGE. 2 µg/lane of Recombinant Human MDH1 His-tag (Catalog # 11632-MH) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 35-38 kDa, under reducing conditions.

BACKGROUND

Malate Dehydrogenase 1 (MDH1), also known as aromatic alpha-keto acid reductase, belongs to the family of nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenases and is a key enzyme in the malate-aspartate shuttle where it plays a critical role in the regulation of metabolic activity in humans (1, 2). MDH1 is an active homodimer of monomers that each contain the characteristic conserved Rossman fold, similar NAD binding sites, and dimeric quaternary structure of MDH enzymes (3). MDH is ubiquitously expressed in cells in two separate isoforms that differ in cellular localization and share only 26% sequence homology (3). While MDH2 is a mitochondrial form involved in the regulation of mitochondrial NAD levels within the citric acid cycle, MDH1, the cytosolic form, plays an important role in the regulation of cytosolic NAD levels. Increased cytosolic NAD levels are necessary for maintaining enhanced glycolysis of proliferating cancer cells (4) and MDH1 is overexpressed in tumors and correlated with poor prognosis (5-8). In addition, MDH1 has been found to serve as a potential marker in several diseases with inflammation and deficiency of MDH1 is associated with early onset severe encephalopathy and associated with acute liver failure (2, 9-11) making it a metabolic therapeutic target of interest with several potential applications (1, 3, 6, 12, 13).

References:

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