

SPECIFICATION SHEET

Product Name: Malate Dehydrogenase
Catalog # MD01D-E311H

Product Description: Recombinant research grade malate dehydrogenase from a microorganism. This enzyme is sold as a lyophilized white powder.

EC 1.1.1.37

Storage and Stability: Product can be stored at 2-8°C for transportation process up to ten days but long-term storage should be at -20°C.

Alternative Names	: MDH, NAD-L-malate dehydrogenase, malic acid dehydrogenase.
Unit Definition	: One unit causes the oxidation of one micromole of NADH per minute at 30°C pH 8.0.
Appearance	: White amorphous powder, lyophilized.
Molecular Weight	: 33.4 kDa
Activity	: ≥500U / ≥2500U
Pack Size	: 500U / 2500U
Scientific Background	: Malate dehydrogenase is an enzyme that uses the reduction of NAD ⁺ to NADH to reversibly catalyze the oxidation malate to oxaloacetate ¹ . It functions in various metabolic pathways including in the Krebs cycle where it protects against oxidative stress as oxaloacetate binds free radicals ² . Release of malate dehydrogenase into the serum is an indicator of tissue damage and has been implicated as a biomarker of several diseases ^{3,4} . Applications of this enzyme include enzymatic determination of L-malate and of glutamate oxaloacetate transaminase (GOT) in clinical analysis.
Reference	1. Minárik P., et al. Malate dehydrogenases-structure and function. Gen Physiol Biophys. 2002 Sep;21(3):257-65. PMID: 12537350. 2. Takahashi-Íñiguez T., et al. Function, kinetic properties, crystallization, and regulation of microbial malate dehydrogenase. J Zhejiang Univ Sci B. 2016 Apr;17(4):247-61. PMCID: PMC4829630. 3. Shelli S., et al. Assessment of Emerging Biomarkers of Liver Injury in Human Subjects, Toxicological Sciences, Volume 132, Issue 2, April 2013, Pages 276-283. 4. Ma YC, et al: Urinary malate dehydrogenase 2 is a new biomarker for early detection of non small-cell lung cancer. Cancer Sci. Jun;112(6):2349-2360, 2021.

Malate Dehydrogenase Activity Assay Protocol

Reaction Components

- A. 0.1 M K-phosphate buffer, pH 7.5
- B. 15 mM Oxaloacetate solution [2 mg oxaloacetic acid (Sigma, 04126-1G)/mL of ice-cold K-phosphate buffer (A). This reagent is rather unstable and should be stored in an ice-bath during use] (Should be prepared fresh)
- C. 6.0 mM NADH solution (Carbosynth, NN12622, should be prepared fresh)
- D. 0.1 M Enzyme dilution buffer (K-phosphate buffer, pH 7.5 containing 0.2% BSA)

- Step 1** Reconstitute lyophilized enzyme powder with water to desired concentration.
- Step 2** Dilute the reconstituted enzyme preparation to 0.05-0.5 U/mL with the enzyme diluent (D), immediately before the assay.
- Step 3** Prepare the following reaction mixture in a cuvette (d=1.0 cm) and equilibrate at 30°C for about 5 minutes.

2.8 mL	K-phosphate buffer	(A)
0.1 mL	Oxaloacetate solution	(B)
0.1 mL	NADH solution	(C)

- Step 4** Add 0.05 mL of the enzyme solution to the reaction mixture and mix gently.
- Step 5** Record the decrease of optical density at 340 nm for 5 minutes in a spectrophotometer. Keep the reaction at a temperature of 30°C.
- Note: Blank the spectrophotometer with water
- Step 6** Calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test)
- Step 7** Measure the blank rate (ΔOD blank) by the same method as the test except that the enzyme diluent (D) is added instead of the enzyme solution.

Unit definition: One unit causes the oxidation of one micromole of NADH under the conditions described above.

Calculation: Activity can be calculated by using the following formula:

$$\text{Volume Activity (U/mL)} = \frac{\Delta OD/\text{min} (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times V_t \times df}{(6.22 \times 1.0 \times V_s)} = \Delta OD/\text{min} \times 9.81 \times df$$

$$\text{Weight Activity (U/mg)} = (\text{U/mL}) \times 1/C$$

V_t : Total volume (3.05 mL)

V_s : Sample volume (0.05 mL)

6.22 : Millimolar extinction coefficient of NADH ($\text{cm}^2/\text{micromole}$)

1.0: Light path length (cm)

df : Dilution factor

C: Enzyme concentration (mg/mL)