Malate Dehydrogenase Activity Assay Kit (WST-8)

L1507463

Storage: -20℃. Protect from light.

Introduction

Malate dehydrogenase (MDH) is an intracellular oxidoreductase widely present in animals, plants, and microorganisms. As a key enzyme in biological sugar metabolism, it catalyzes the interconversion between malate and oxaloacetate. MDH plays important roles in various cellular physiological activities, including mitochondrial energy metabolism, the malate-aspartate shuttle system, and reactive oxygen species metabolism in plants.

MDH exists in multiple isozymes, with two main isoforms in eukaryotic cells: mitochondrial MDH (m-MDH) and cytosolic MDH (c-MDH). m-MDH, located in mitochondria, primarily catalyzes the dehydrogenation (oxidation) of malate and is one of the essential enzymes in the tricarboxylic acid (TCA) cycle, playing a crucial role in the complete oxidation or interconversion of nutrients in vivo. c-MDH is localized in the cytoplasm and mainly catalyzes the hydrogenation (reduction) of oxaloacetate. It is a key enzyme in the malate-aspartate shuttle system, ensuring that NADH generated during glycolysis in the cytoplasm enters mitochondria for complete oxidation and energy production. MDH plays a vital role in maintaining normal life activities. Clinical studies have shown that serum MDH activity increases significantly in the early stages of myocardial infarction and viral hepatitis. Additionally, elevated serum MDH activity to varying degrees has been observed in kidney diseases, rheumatoid and rheumatic diseases, traumatic shock, hematological diseases, etc. Therefore, the determination of MDH activity is of great significance for understanding related metabolic states as well as for the diagnosis and prevention of clinically relevant diseases.

The detection principle of this kit is as follows: L-malate is oxidized to oxaloacetate by malate dehydrogenase, during which NAD^+ is reduced to NADH. The generated NADH then reduces WST-8 to an orange-yellow formazan dye in the presence of the electron coupling reagent 1-methoxy-5-methylphenazinium methyl sulfate (1-mPMS). This formazan product exhibits a maximum absorption peak at approximately 450 nm. The amount of formazan generated is directly proportional to the malate dehydrogenase activity in the sample. With a sample volume of 20 μL, this kit can detect MDH activity as low as 13 μU and shows a strong linear relationship within the activity range of 0.67–33.3 U/L. The kit includes a standard solution of NADH, the product generated by MDH catalysis, allowing the establishment of a standard curve for calculating the malate dehydrogenase activity in samples.

Kit Contents

L1507463	Component	50 T	100 T	Storage conditions
L1507463A	Lysis Buffer	10 mL	20 mL	-20℃.
L1507463B	Assay Buffer	10 mL	20 mL	-20℃.
L1507463C	Chromogenic Solution	100 μL	200 μL	-20℃.Store in the dark.
L1507463D	L-malate	250 μL	500 μL	-20℃.Store in the dark.
L1507463E	Coenzyme	200 μL	400 μL	-20℃.Store in the dark.
L1507463F	NADH	5 mg	5 mg×2	-20℃.Store in the dark.

Usage Protocol

1. Sample Preparation

1) Preparation of Blood Samples:

For serum samples: Allow whole blood to clot at room temperature for 30 minutes to 2 hours. Centrifuge at approximately 1000-2000 × g for 10 minutes at 4°C and collect the supernatant.

For plasma samples: Collect whole blood using heparin or EDTA as an anticoagulant. Centrifuge at approximately 1000-2000 × g for 10 minutes at 4°C and collect the supernatant.

2) Preparation of Cell Samples:

For cultured adherent cells: Wash the cells once with PBS; For cultured suspension cells: Centrifuge to collect the cells and wash them once with PBS. Add Lysis Buffer at a ratio of 200 μL per 1 million cells. Pipette appropriately to mix and incubate on ice for 10 minutes for complete lysis. Centrifuge at 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent assays.

3) Preparation of Tissue Samples:

Add Lysis Buffer at a ratio of 100 μL per 10 mg of tissue. Homogenize the tissue using a homogenizer on ice. Centrifuge at approximately 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent assays.

4) Preparation of Cell Culture Supernatant Samples:

For adherent cells: Directly collect the culture medium; For suspension cells: Centrifuge the culture and collect the supernatant.

All the above procedures must be performed at 4°C or on ice. Prepared cell or tissue samples can be stored at -20°C or -80°C if not assayed immediately.

2. Establishment of NADH Standard Curve

Add 705 μL of Assay Buffer to the 5 mg of NADH provided in the kit, fully dissolve and mix to obtain a 10 mM NADH standard solution. Except for the portion to be used immediately, aliquot the remaining NADH standard solution appropriately and store at –80 °C protected from light. Avoid repeated freeze- thaw cycles.

Pipette 10 μL of the NADH standard solution (10 mM) and add it to 90 μL of lysis buffer or

assay buffer (use lysis buffer for lysed cell or tissue samples; use assay buffer for samples such as blood or supernatant that do not require processing) to prepare a 1 mM NADH standard working solution. Then add 0, 2, 4, 8, 12, 16, and 20 µL of the 1 mM NADH standard working solution into the standard wells of a 96- well plate, and bring the volume in each well to 20 µL by adding the corresponding lysis buffer or assay buffer. The resulting standard curve concentrations are 0, 100, 200, 400, 600, 800, and 1000 µM.

3. Preparation of WST-8 Working Solution

Prepare the WST-8 Working Solution according to the table below. Note: This procedure must be performed on ice.

Samples	1	10	20	50
Assay Buffer	69 µL	690 µL	1380 µL	3450 µL
Coenzyme	4 µL	40 µL	80 µL	200 µL
Chromogenic Solution	2 µL	20 µL	40 µL	100 µL
L-Malate	5 µL	50 µL	100 µL	250 µL
Total volume	80 µL	800 µL	1600 µL	4000 µL

Note: When the sample background may interfere with detection, a sample background control well must be set up in parallel. Add the Color Developer Working Solution prepared without the substrate (replace the 5 µL of substrate with Assay Buffer). When calculating results, subtract the absorbance reading of the sample background control well from that of the sample well.

4. Sample Assay

1) Add 1–20 µL of sample or diluted sample to the sample wells of a 96-well plate. Then add the corresponding Assay Lysis Buffer or Assay Buffer to the sample wells to bring the volume to 20 µL. At the same time, set up wells containing only lysis buffer or assay buffer as blank control wells (use lysis buffer if detecting cell or tissue samples that require lysis; use assay buffer if detecting samples such as blood or supernatant that do not require lysis).
2) Add 80 µL of WST-8 Color Developer Working Solution to each well and mix. Immediately measure A_{450} using a suitable microplate reader or micro-volume UV-Vis spectrophotometer, and record this as the 0-minute reading A_1 .
3) React at 37 °C for 10–30 minutes, record the reaction time as T, measure A_{450}, and record it as A_2 . The signal increase depends on the amount of NADH generated by MDH catalysis: $\Delta A = A_2 - A_1$.

Note: To achieve optimal detection results, the reaction time can be adjusted according to the MDH activity in the sample, but it must be ensured that the readings fall within the standard curve range. For samples with higher MDH activity, it is recommended to set the total measurement time to 20 or 30 minutes, with corresponding measurement intervals of 2 or 5 minutes; for samples with lower MDH activity, the total measurement time can be extended to 1–2 hours, with corresponding measurement intervals of 10 or 20 minutes. Alternatively, continuous measurement can be performed for 30 minutes, reading every 1 or 2 minutes, and

finally, the data before the linear time point can be taken for analysis and calculation.

5. Calculation of Results

Establish the NADH standard curve. By substituting ΔA into the standard curve, the concentration of NADH (B) generated by MDH catalysis in the sample during the reaction time can be calculated. The formula for calculating MDH activity is as follows: MDH Activity (U/L) = B × n / T

Note: B is the NADH concentration (μM) determined from the standard curve; n is the total dilution factor of the sample; T is the reaction time (minutes).

Definition of Malate Dehydrogenase Enzyme Activity Unit: One enzyme activity unit (U) is defined as the amount of enzyme that catalyzes the formation of 1 μmol of NADH per minute at 37 °C.

Precautions

1. All components must be completely thawed and equilibrated to room temperature before use, otherwise the detection results may be affected. The color developer, substrate, and NADH must be protected from light during storage and use.
2. NADH is relatively unstable. After being prepared as a solution, it should be aliquoted appropriately, stored at –80 °C, and used as soon as possible. If the standard curve appears unsatisfactory, it is likely due to degradation of the NADH standard. During each assay, after taking out the aliquoted NADH standard, keep it protected from light and use it promptly. Repeated freeze- thaw cycles are prohibited.
3. If serum or similar samples are stored at 4 °C, the storage time should not exceed two weeks; otherwise, the accuracy of the detection results may be affected. Generally, serum samples are recommended to be stored at –20 °C, and storage at –80 °C is even better.