PRODUCT INFORMATION & MANUAL

Malate Dehydrogenase (MDH) Activity Assay Kit (Colorimetric)

NBP3-25898

For research use only.
Not for diagnostic or therapeutic procedures.

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Malate Dehydrogenase (MDH) Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25898
Method: Colorimetric method
Specification: 50Assays **(**Can detect 48 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 0.01 U/mL
Detection range: 0.01-1.2 U/mL

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used for detection of Malic Dehydrogenase (MDH) activity in serum and plasma samples.

Detection significance

MDH has close links with various important ways of plant metabolism. It plays an important role in the process of Mal / OAA (malic acid/oxaloacetic acid) and Mal / Asp (malic acid / aspartic acid) material transporting and energy transporting. In photorespiration, MDH provides NAD^+ for the oxidation of Gly (glycine). In mitochondria, MDH is one of the regulatory enzymes that decide the running speed of TCA (tricarboxylic acid cycle). In cytosol, MDH is related to the branch of pyruvic acid. Therefore, MDH system is not only a good system to research the regionalization and regulation of enzyme, but also facilitates the study of the relationship between organelles and many developmental problems.

Detection principle

MDH catalyzed redox reactions were accompanied by a decrease in absorbance at 340nm. MDH activity can be calculated indirectly by measuring the change rate in absorbance (ΔA/min) at 340 nm.

Kit component

	Components	**Specifications**	**Storage**
Reagent 1	Liquid	12 mL ×5 vials	-20℃, 12 months
Reagent 2(A)	Powder	3 vials	-20℃, 12 months
Reagent 2(B)	Diluent	0.5 mL ×3 vials	-20℃, 12 months
Preparation of reagent 2 application solution: Dissolve a vial of reagent 2(A) with a vial of reagent 2(B) and mix fully. Prepare the fresh solution before use.			
Reagent 3(A)	Powder	2 vials	-20℃, 12 months
Reagent 3(B)	Diluent	1 mL ×2 vials	-20℃, 12 months
Preparation of reagent 3 application solution: Dissolve a vial of reagent 3(A) with a vial of reagent 3(B) and mix fully.			
Preparation of working solution: Mix the reagent 1, reagent 2 application solution, reagent 3 application solution at the ratio of 50: 1: 1 fully. Prepare the needed amount fresh solution before use.			

Experimental instruments

Tube, Micropipettor, Vortex mixer, Incubator, Spectrophotometer (340 nm)

Sample preparation

Serum (plasma) sample: Detect directly.

Operation steps

1. Preheat the spectrophotometer for 30 min, then adjust the wavelength at 340 nm and set the spectrophotometer to zero with distilled water. (Prepare two quartz cuvette, one for setting zero, the other for measuring).
2. Preheat the **working solution** at 37℃ for more than 3 min.
3. Operation table

	Sample tube	Blank tube
Sample (μL)	100	
Double-distilled water (μL)		100
Working solution (μL)	1000	1000
Mix fully immediately, measure OD value of each tube at 340 nm with 0.5 cm optical path cuvette. The OD value of 20 seconds and 80 seconds were recorded as A1 and A2, respectively. Calculate the ∆A=A1-A2.		

[Note]

(1) Blank tube only need to be done 1-2 times.

(2) If $\triangle A_{Sample}/min < 0.05$, please increase the concentration of sample and test again, otherwise the result will be influenced.

(3) If $\Delta A_{Sample}/min > 0.3$, please dilute the sample and test again, otherwise the result will be influenced.

(4) It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

Calculation of results

Definition: The amount of MDH in 1 mL of serum or plasma that catalyze the reaction of 1 μmol of substrate per minute is defined as 1 unit.

$$\text{MDH Activity } (U/mL) = \frac{\triangle A_{Sample} - \triangle A_{Blank}}{6.2^{*} \times \text{optical path } (0.5\ cm)} \times \frac{OD_{Sample}\text{-}OD_{Blank}}{OD_{Standard}\text{-}OD_{Blank}} \times \frac{\text{Total volume of reaction solution}(1.1\ mL)}{\text{Sample volume } (0.1\ mL)} \times \text{Dilution factor of sample before tested}$$

6.2*: Micromole extinction coefficient of substrate

Note:

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.