Technical Bulletin

Malate Dehydrogenase Assay Kit

Catalogue number MAK512

Product Description

Malate dehydrogenase (MDH) is an enzyme which reversibly catalyzes the oxidation of L-malate to oxaloacetate in the presence of NAD. There are 2 isoforms in eukaryotic cells: MDH1 and MDH2. MDH1 is found in the cytoplasm and plays a key part in the malate aspartate shuttle for transporting malate into the mitochondria. MDH2 is a mitochondrial enzyme which participates in the TCA cycle that reversibly converts L-malate into oxaloacetate. Higher MDH activities are found in some neurodegenerative diseases such as Alzheimer's disease.

The Malate Dehydrogenase Assay Kit is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity.

The linear detection range of the kit is 0.5 to 65 U/L. The kit is suitable for malate dehydrogenase determination in biological samples such as plasma, serum, erythrocytes, tissue and culture media.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK512A
- Enzyme A 120 µL
  Catalogue Number MAK512B
- Enzyme B 120 µL
  Catalogue Number MAK512C
- NAD/MTT 1 mL
  Catalogue Number MAK512D
- Substrate 600 µL
  Catalogue Number MAK512E
- Calibrator 1.5 mL
  Catalogue Number MAK512F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- Phosphate Buffered Saline (Catalogue Number PPB006 or equivalent)
- Dounce Tissue Grinder Set (Catalogue Number D9063 or equivalent)
- 1.5 mL microcentrifuge tubes.

Precautions and Disclaimer

For Research use only. Not for diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Assays can be executed at any desired temperature 25 °C or 37 °C, equilibrate all components to desired temperature (37 °C is recommended) prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Serum and plasma are assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize 50 mg of tissue in ~200 µL of buffer containing 50 mM potassium phosphate (pH 7.5)
3. Centrifuge at 14,000 × g for 10 minutes at 4 °C.
4. Remove supernatant for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × g for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5)
4. Centrifuge at 14,000 × g for 10 minutes at 4 °C.
5. Remove supernatant for assay.

All Samples can be stored at –20 °C to –80 °C for at least one month.

Assay Reaction

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to wells should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Set the plate reader for the desired assay temperature 25 °C or 37 °C.
2. Transfer 100 µL of purified water (OD_{H2O}) and 100 µL of Calibrator (OD_{CAL}) solution into separate wells of a clear flat bottom 96-well plate.
3. Transfer 20 µL H_2O into one well, this will be the blank. Transfer 20 µL of each Sample into separate wells.

Working Reagent Preparation

For each well, prepare 89 µL of Working Reagent to Table 1.

Table 1.

Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	74 µL
NAD/MTT	8 µL
Substrate	5 µL
Enzyme A	1 µL
Enzyme B	1 µL

Add 80 µL Working Reagent to all Samples and blank wells. Tap plate briefly to mix.

Measurement

Read the optical density (OD) of each well at 565 nm at 10 minutes and again at 30 minutes (OD_{10} and OD_{30} respectively). Alternatively, using the plate reader's kinetic mode, monitor the OD for 30 minutes.

Results

1. Subtract the OD_{10} from OD_{30} for each sample and blank well to compute the ΔOD_S and ΔOD_B values respectively.
2. MDH activity can then be calculated as follows:

$$\text{MDH Activity} = \frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{MTT} \times l} \times \frac{\text{Reaction Vol (µL)}}{\text{t (min) x Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H2O}} \times \frac{273}{\text{t (min)}} \times n \text{ (U/L)}$$

Where:

ε_{mtt} is the molar absorption coefficient of reduced MTT.

l is the light pathlength which is calculated from the calibrator.

OD_{CAL} and OD_{H20} are OD_{565nm} (OD_{10}) values of the Calibrator and water.

t is the difference in time between readings (20 minutes).

Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively.

n is the dilution factor if applicable.

Note: If sample MDH activity exceeds 65 U/L, dilute samples in water and repeat the assay. For samples with MDH activity < 1 U/L, the incubation time can be extended to 2 hours.

Unit definition: 1 Unit (U) of MDH will catalyze the conversion of 1 µmole of oxaloacetate and NADH per minute at pH 7.5.

Figure 1.
Example of Raw Kinetics Data

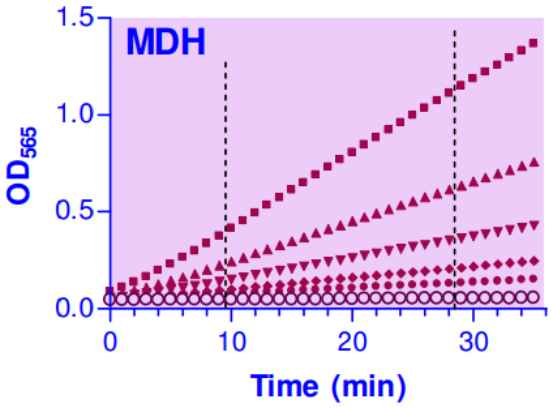
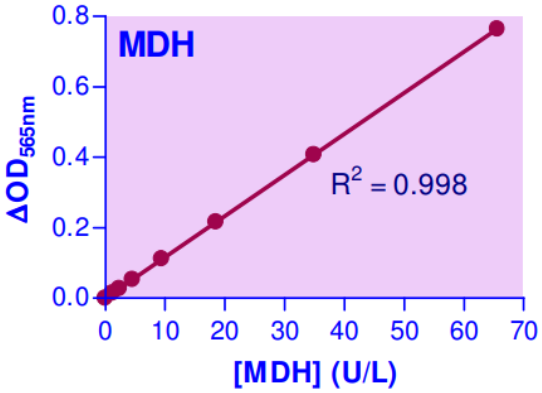


Figure 2.

Example of MDH Activity (20 min, 37 °C)



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