3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Malate Dehydrogenase Assay Kit

Catalog Number **MAK196**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Malate dehydrogenase (MDH; EC 1.1.1.37) is an NAD oxidoreductase that catalyzes the NAD/NADH-dependent interconversion of malate and oxalacetate. This reaction is critical to maintain the malate/aspartate shuttle across the mitochondrial membrane and Krebs tricarboxylic acid cycle in the mitochondrial matrix.[1,2] Increased MDH activity has been observed in neurodegenerative diseases such as Alzheimer's disease.[3]

The Malate Dehydrogenase Assay Kit provides a simple and sensitive procedure for measuring MDH activity in a variety of tissues, cell cultures, and isolated mitochondria. MDH activity is determined by generating a product with absorbance at 450 nm proportional to the enzymatic activity present. One unit of MDH is the amount of enzyme that generates 1.0 μmole of NADH per minute at 37 °C and pH 9.5.

Components

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

MDH Assay Buffer Catalog Number MAK196A	20 mL
MDH Substrate Catalog Number MAK196B	1 vl
MDH Enzyme Mix Catalog Number MAK196C	1 vl
MDH Developer Catalog Number MAK196D	1 vl
NADH Standard Catalog Number MAK196E	1 vl
MDH Positive Control Catalog Number MAK196F	1 vl

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Mitochondria Isolation Kit (optional for mitochondria samples, Catalog Number MITOISO1 for tissue, MITOISO2 for cells, MITOISO3 for yeast, or equivalent)
- Saturated ammonium sulfate (~4.1 M at room temperature, optional for removing small molecules)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

MDH Assay Buffer – Store the buffer at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use.

MDH Substrate – Reconstitute with 220 μL of MDH Assay Buffer. Mix well by pipetting and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

MDH Enzyme Mix – Reconstitute with 220 μL of MDH Assay Buffer. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

MDH Developer – Reconstitute with 1.05 mL of water. Mix well by pipetting and store at –20 °C. Use within 2 months of reconstitution.

NADH Standard – Reconstitute with 400 µL of water to prepare a 1.25 mM (1.25 nmole/µL) NADH Standard Solution. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

MDH Positive Control – Reconstitute with 400 µL of MDH Assay Buffer. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standard Curve Preparation
Add 0, 2, 4, 6, 8, and 10 µL of the 1.25 mM NADH Standard Solution into a 96 well plate, generating 0 (blank), 2.5, 5, 7.5, 10, and 12.5 nmole/well standards. Add MDH Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (1×10^6) can be homogenized in ice cold MDH Assay Buffer. Place on ice for 10 minutes. Centrifuge at 10,000 × *g* for 5 minutes at 2–8 °C and collect the supernatant.

Note: When analyzing MDH activity in mitochondria, it is recommended to isolate the mitochondria from fresh tissue or cells.

Add 1–50 µL sample per well and bring the final volume to 50 µL with MDH Assay Buffer.

Note: For unknown samples, test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the MDH Substrate. The Sample Blank readings can then be subtracted from the sample readings.

For the positive control (optional), add 1–10 µL of the MDH Positive Control solution to wells and adjust the volume to 50 µL with the MDH Assay Buffer.

Note: Small molecules in some tissue samples such as heart may interfere with the assay. To remove small molecules after homogenizing, it is suggested to use an ammonium sulfate precipitation method. Pipette 50 µL of homogenate into a fresh tube, add 2× volume of saturated ammonium sulfate (~4.1 M at room temperature) and keep on ice for 20 minutes. Centrifuge at 10,000 × *g* for 5 minutes, remove and discard the supernatant, and resuspend the pellet to the original volume with MDH Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Standards and Samples	Sample Blank
MDH Assay Buffer	36 µL	38 µL
MDH Enzyme Mix	2 µL	2 µL
MDH Developer	10 µL	10 µL
MDH Substrate	2 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.
3. Incubate the plate at 37 °C. Take the initial measurement. Measure the absorbance at 450 nm [$(A_{450})_{initial}$] at the initial time ($T_{initial}$).
4. Continue to incubate the plate at 37 °C taking measurements (A_{450}) every 5 minutes for 10–30 minutes.
   Note: Incubation time depends on the activity of MDH in the samples.
5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
6. The final absorbance measurement [$(A_{450})_{final}$] for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see step 5). The time of the penultimate reading is T_{final}.
   Notes: It is essential that $(A_{450})_{final}$ falls within the linear range of the standard curve.

   The NADH Standards can be read at the end of the incubation time.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{450})_{final}$] obtained for the 0 (blank) NADH Standard from the final measurement [$(A_{450})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate NADH Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in absorbance measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Subtract the Sample Blank ΔA_{450} value from the sample ΔA_{450} reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of NADH (nmole/well) generated by the MDH assay between $T_{initial}$ and T_{final} (S_a).

MDH activity:

$$\text{MDH Activity} = \frac{S_a}{(\text{Reaction Time}) \times S_v}$$

where:

S_a = Amount of NADH (nmole) generated in unknown sample well between $T_{initial}$ and T_{final} from standard curve

Reaction Time = $T_{final} - T_{initial}$ (minutes)

S_v = sample volume (mL) added to well

MDH activity is reported as nmole/min/mL=milliunit/mL. One unit of MDH is the amount of enzyme that generates 1.0 μmole of NADH per minute at 37 °C and pH 9.5.

Sample Calculation:

Amount of NADH (S_a) = 5.84 nmole (from standard curve)

($T_{initial}$) = 3 minutes

(T_{final}) = 32 minutes

Sample volume (S_v) = 0.05 mL

MDH activity in sample well:

$$\text{nmole/min/mL (milliunits/mL)} = \frac{5.84 \text{ nmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 0.05 \text{ mL/well}} = 4.03$$

References

1. Minárik, P. et al., Malate dehydrogenases–structure and function. Gen. Physiol. Biophys., **21**, 257–265 (2002).
2. Muramatsu, H. et al., A new family of NAD(P)H-dependent oxidoreductases distinct from conventional Rossmann-fold proteins. J. Biosci. Bioeng., **99**, 541–547 (2005).
3. Zahid, S. et al., Differential expression of proteins in brain regions of Alzheimer's disease patients. Neurochem. Res., **39**, 208–215 (2014).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

GD,KVG,LS,MAM 10/14-1