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PicoProbe™ Malate Dehydrogenase Activity Assay Kit (Fluorometric)

05/17

(Catalog #K538-100; 100 assays; Store at -20°C)

I. Introduction:

Malate Dehydrogenase (MDH) (EC 1.1.1.37) reversibly converts L-Malate into oxaloacetate in the presence of NAD^+. In eukaryotic cells, malate dehydrogenase has 2 isoforms, MDH1 and MDH2. MDH1 is cytosolic and participates in the malate-aspartate shuttle, which transports malate into the mitochondria. Malate will then be utilized for the generation of ATP. MDH2, on the other hand, is a mitochondrial enzyme that participates in the citric acid cycle. MDH2 activity is increased in some neurodegenerative diseases such as Alzheimer's disease, and abnormal MDH activity in serum can serve as a diagnostic tool for severe liver damage (e.g. Hepatocellular carcinoma). Biovision's PicoProbe™ Malate Dehydrogenase Activity Assay Kit (Fluorometric) provides a quick and easy method for monitoring MDH activities in a wide variety of samples. In this assay, MDH converts malate into intermediate products, which will further react with PicoProbe™ forming a fluorophore (Ex/Em=535/587nm). The kit is simple, sensitive and high-throughput adaptable and can detect as low as 40 µU of MDH activity.

$$\text{Malate} \xrightarrow{\text{Malate Dehydrogenase}} \text{Intermediate} \xrightarrow{\text{PicoProbe}^{TM}} \text{Fluorescence (Ex/Em=535/587nm)}$$

II. Applications:

- Measurement of malate dehydrogenase activity in various tissues/cells
- Analysis of citrate acid cycle and malate-aspartate shuttle

III. Sample Type:

- Animal tissues: heart, liver, muscle, etc.
- Purified mitochondria
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K538-100	Cap Code	Part Number
MDH Assay Buffer	25 ml	WM	K538-100-1
MDH Substrate	1 vial	Blue	K538-100-2
PicoProbe™	450 µl	Red	K538-100-3
MDH Developer	1 vial	Green	K538-100-4
NADH Standard	1 vial	Yellow	K538-100-5
MDH Positive Control	1 vial	Orange	K538-100-6

V. User Supplied Reagents and Equipment:

- 96-well opaque white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **MDH Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **MDH Substrate:** Reconstitute with 220 µl Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.
- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Avoid from light.
- **MDH Developer:** Reconstitute with 220 µl Assay buffer. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **MDH Positive Control:** Reconstitute with 400 µl Assay Buffer to the Positive Control and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VII. Malate Dehydrogenase Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (~10 mg) or cells (~1 x 10^6) with 100 µl ice cold MDH Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x *g* for 5 min. at 4 ºC and transfer the supernatant to a fresh tube. Add 2-50 µl sample per well in a white plate & adjust the volume to 50 µl with MDH Assay Buffer. For mitochondrial MDH activity: isolate the mitochondria from fresh tissue or cells using BioVision's Mitochondria Isolation Kit for Tissue and Cultured Cells (K288-50). Add 2-50 µl of isolated mitochondria per well in a 96-well white plate, adjust the volume to 50 µl with MDH Assay Buffer. For the MDH positive control: dilute the MDH positive control 50X of the stock solution by adding 4 µl of the reconstituted MDH positive into 196 µl of MDH Assay buffer. Take 2-20 µl of MDH Positive Control into desired well(s) and adjust the final volume to 50 µl with MDH Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples exhibiting significant background, prepare parallel sample well(s) as sample background controls.

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2. **NADH Standard Curve:** Dilute the NADH standard to 50 µM (50 pmol/µl) by adding 4 µl of the reconstituted NADH standard into 96 µl of dH_2O. Add 0, 2, 4, 6, 8 and 10 µl of 50 µM NADH Standard into a series of wells in a 96-well white plate to generate 0, 100, 200, 300, 400 and 500 pmol/well of NADH Standard. Adjust the volume to 50 µl/well with MDH Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	*Background Control Mix
MDH Assay Buffer	44 µl	46 µl
MDH PicoProbe™ Developer	2 µl	2 µl
PicoProbe™	2 µl	2 µl
MDH Substrate	2 µl	----

Mix and add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples.
* For background correction, add 50 µl of Background Control Mix (without substrate) to sample background control well(s) and mix well.

4. **Measurement:** Measure Fluorescence (Ex/Em= 535/587 nm) immediately in kinetic mode for 10-60 min. at 37°C.

Note: Incubation time depends on the malate dehydrogenase activity in samples. We recommend measuring the fluorescence in kinetic mode, and choosing two time points (t_1 & t_2) in the linear range to calculate the malate dehydrogenase activity of the samples. The NADH Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the malate dehydrogenase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the NADH Standard Curve to get B pmol of NADH generated during the reaction time ($\Delta t = t_2 - t_1$).

Sample Malate Dehydrogenase Activity = B/(Δt X V) x D = pmol/min/ml = µU/µl

Where: **B** = NADH amount from Standard Curve (pmol)
Δt = reaction time (min)
V = sample volume added into the reaction well (µl)
D = Dilution Factor

Unit Definition: One unit of malate dehydrogenase is the amount of enzyme that generates 1.0 µmol of NADH per min at pH 9.5 at 37°C.

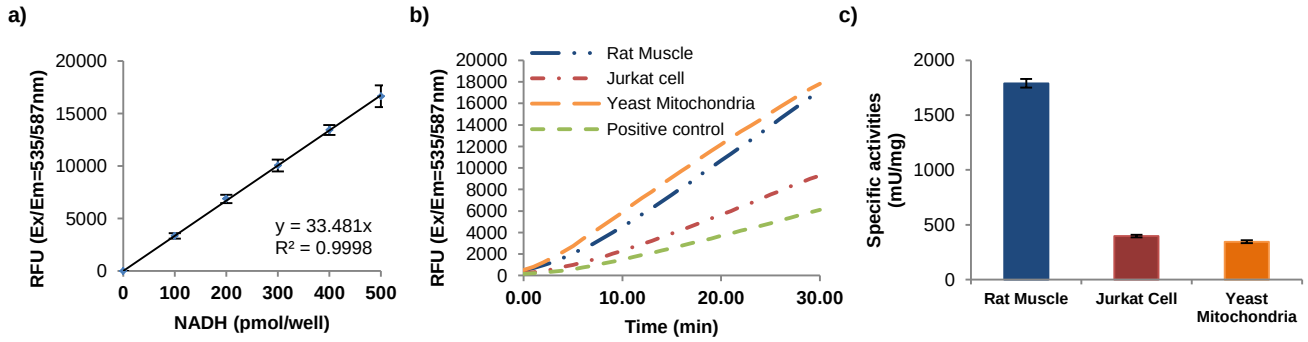

Figure: (a) NADH Standard Curve; (b) Malate Dehydrogenase activities in rat muscle extract (0.011 µg protein), Jurkat cell lysate (0.026 µg protein), Saccharomyces mitochondria (0.05 µg) & MDH positive control; (c) Specific MDH activities in rat muscle extract, Jurkat cell lysate and yeast Mitochondria. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Malate Colorimetric Assay Kit (K637)
Malate Dehydrogenase Activity Colorimetric Assay kit (K654)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Citrate Colorimetric/Fluorometric Assay Kit (K655)
Citrate Synthase Activity Colorimetric Assay Kit (K318)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
α-Ketoglutarate Colorimetric Assay Kit (K677)
Fumarate Colorimetric Assay Kit (K633)
PicoProbe™ Lactate Dehydrogenase Activity Assay kit (K730)
PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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