Malate Dehydrogenase Activity Colorimetric Assay Kit

(Catalog # K654-100; 100 assays; Store at -20 °C)

I. Introduction:
Malate Dehydrogenase (MDH) (EC 1.1.1.37) is an important enzyme which 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 & participates in the malate-aspartate shuttle, which transports malate into mitochondria for utilization in ATP generation whereas MDH2 is a mitochondrial enzyme and part of the citric acid cycle. MDH 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). In BioVision’s Malate Dehydrogenase Activity Assay Kit, MDH reacts with malate to form an intermediate. The generated intermediate reacts with MDH Developer to form a colored product with strong absorbance at 450 nm. The assay is simple, sensitive and can detect less than 0.5 mU of MDH activity in various sample types.

II. Applications:
- Measurement of malate dehydrogenase activity in various tissues/cells
- Analysis of citric acid cycle and malate-aspartate shuttle

III. Sample Type:
- Animal tissues such as liver, heart, muscle, etc.
- Cell culture: Adherent or suspension cells
- Mitochondria

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K654-100</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDH Assay Buffer</td>
<td>20 ml</td>
<td>WM</td>
<td>K654-100-1</td>
</tr>
<tr>
<td>MDH Substrate (Lyophilized)</td>
<td>1 vial</td>
<td>Violet</td>
<td>K654-100-2</td>
</tr>
<tr>
<td>MDH Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
<td>Green</td>
<td>K654-100-3</td>
</tr>
<tr>
<td>MDH Developer (Lyophilized)</td>
<td>1 vial</td>
<td>Red</td>
<td>K654-100-4</td>
</tr>
<tr>
<td>NADH Standard (Lyophilized)</td>
<td>1 vial</td>
<td>Yellow</td>
<td>K654-100-5</td>
</tr>
<tr>
<td>MDH Positive Control (Lyophilized)</td>
<td>1 vial</td>
<td>Orange</td>
<td>K654-100-6</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:
Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:
- **MDH Assay Buffer**: Warm to room temperature before use. Store at either 4 °C or -20 °C.
- **MDH Substrate**: Reconstitute with 220 µl Assay Buffer. Store at -20 °C. Keep on ice while in use. Use within two months.
- **MDH Enzyme Mix**: Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20 °C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **MDH Developer**: Reconstitute with 1.05 ml dH2O. Pipette up and down to dissolve completely. Store at -20 °C. Use within two months.
- **NADH Standard**: Reconstitute with 400 µl dH2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Aliquot & store at -20 °C. Keep on ice while in use. Use within two months.
- **MDH Positive Control**: Reconstitute with 400 µl Assay Buffer and mix thoroughly. Aliquot and store at -20 °C. Keep on ice while in use. Use within two months.

VIII. 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. Keep on ice for 10 min. Centrifuge at 10,000 g for 5 min. at 4 °C and collect the supernatant. Add 1-50 µl sample per well & adjust the volume to 50 µl with MDH Assay Buffer. To check mitochondrial MDH activity, isolate mitochondria from fresh tissues or cells using BioVision Mitochondrial Isolation Kit for Tissue & Cultured Cells (K288-50). Add 1-50 µl of isolated mitochondria per well & adjust the volume to 50 µl with MDH Assay Buffer. Add 1-10 µl of MDH Positive Control into desired well(s) & adjust the 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 having background, prepare parallel sample well(s) as sample background control(s).
- c. Small molecules in some tissue samples such as heart may interfere with the assay. To remove small molecules, we recommend using ammonium sulfate method to precipitate the enzymes. Transfer tissue homogenate (50 µl) to a clean centrifuge tube & add 2
Yeast mitochondria (1.2 μg), Jurkat cell lysate (0.6 μg), yeast mitochondrial lysate (1.2 μg) & MDH Positive Control (b). Referenced MDH Activity in Jurkat cell lysate, rat muscle extract and yeast mitochondrial lysate (c). Assays were performed following the kit protocol.

IX. Related Products:
- Malate Colorimetric Assay Kit (K637)
- Fumarate Colorimetric Assay Kit (K633)
- Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
- PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
- Citrate Colorimetric/Fluorometric Assay Kit (K655)
- Oxaloacetate Colorimetric/Fluorometric Assay kit
- Citrate Synthase Activity Colorimetric Assay Kit (K318)
- Isocitrate Colorimetric Assay Kit (K656)
- Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
- Isocitrate Dehydrogenase Activity Colorimetric Assay Kit (K756)
- Alpha-Ketoglutarate Colorimetric Assay Kit (K677)
- Aconitase Activity Colorimetric Assay Kit (K716)

**FOR RESEARCH USE ONLY! Not to be used on humans.**