ENZYMATIC TEST KIT FOR THE DETERMINATION OF L-MALIC ACID IN GRAPE JUICE AND WINE

PRODUCT
Product no. 4A165, for 100 tests, for in vitro use only.

PRINCIPLE OF MEASUREMENT
L-malic acid is found in grape juice and wine and is determined enzymatically according to the following equations:

\[
\text{MDH} \quad \text{L-malate} + \text{NAD}^+ \quad \leftrightarrow \quad \text{Oxaloacetate} + \text{NADH} + \text{H}^+
\]

L-malic acid is oxidised by nicotinamide adenine dinucleotide (NAD) to oxaloacetate using L-malate dehydrogenase (MDH) enzyme as a catalyst. The equilibrium does not favour formation of oxaloacetate and so oxaloacetate is removed by a trapping enzyme. The amount of NADH formed is measured at 340 nm and is stoichiometrically related to the amount of L-malate consumed. In this method, glutamate oxaloacetate transaminase (GOT) is used as the trapping enzyme. In the presence of L-glutamate, the oxaloacetate is irreversibly converted to L-aspartate.

\[
\text{GOT} \quad \text{Oxaloacetate} + \text{L-glutamate} \quad \rightarrow \quad \text{L-aspartate} + \alpha\text{-ketoglutarate}
\]

CONTENTS
The kit includes the following reagents:

<table>
<thead>
<tr>
<th>Reagent No.</th>
<th>Reagent</th>
<th>Preparation</th>
<th>Quantity</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer</td>
<td>Nil</td>
<td>2 x 53 mL</td>
<td>2 years at 4°C</td>
</tr>
<tr>
<td>2</td>
<td>NAD</td>
<td>Add 22.0 mL of distilled water, mix to dissolve</td>
<td>22.0 mL</td>
<td>2 years at 4°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(diluted: 1 year at 4°C, 2 years at -20°C)</td>
</tr>
<tr>
<td>3</td>
<td>GOT</td>
<td>Swirl gently before use</td>
<td>1.3 mL</td>
<td>2 years at 4°C</td>
</tr>
<tr>
<td>4</td>
<td>MDH</td>
<td>Swirl gently before use</td>
<td>1.3 mL</td>
<td>2 years at 4°C</td>
</tr>
<tr>
<td>5</td>
<td>Standard</td>
<td>Nil</td>
<td>3.3 mL</td>
<td>2 years at 4°C</td>
</tr>
</tbody>
</table>

The shelf life of Reagents 1 & 2 can be extended by placing aliquots in a freezer.

Do not freeze enzyme reagents 3 & 4.

Failure to store reagents at the recommended temperature will reduce their shelf life. For concentration of Standard, refer to label on bottle.

SAFETY
- Wear safety glasses
- Reagent 1 is mildly corrosive
- Do not ingest Buffer or Standard as they contain sodium azide as a stabilizer

PROCEDURE
Operating Parameters
- Wavelength: 340 nm
- Cuvettes: 1cm, quartz, silica, methacrylate or polystyrene
- Temperature: 20 – 25°C
- Final volume in cuvette: 2.22 mL
- Zero: against air without cuvette in light path
SAMPLE PREPARATION
Samples should be diluted with distilled water to ensure that the concentration in the assay solution is no more than 0.4 g/L. For samples with less than 2 g/L of L-Malic acid, a 1 in 5 dilution is sufficient. As a general guide, further dilution is required if the absorbance reading at A2 is greater than 1 absorbance unit.

Undiluted red wines or highly coloured undiluted juice samples will require decolourisation. To decolourise, add approximately 0.1 g of PVPP to 5 mL of sample in a test tube. Shake well for about 1 minute. Clarification is achieved by settling, centrifugation, or by filtering through Whatman No. 1 filter paper.

SAMPLE ANALYSIS
a. Pipette the following volumes of reagents into the cuvettes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank assay</th>
<th>Standard assay</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Buffer</td>
<td>1.00 mL (1000 µL)</td>
<td>1.00 mL (1000 µL)</td>
<td>1.00 mL (1000 µL)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.00 mL (1000 µL)</td>
<td>0.90 mL (900 µL)</td>
<td>0.90 mL (900 µL)</td>
</tr>
<tr>
<td>2. NAD</td>
<td>0.20 mL (200 µL)</td>
<td>0.20 mL (200 µL)</td>
<td>0.20 mL (200 µL)</td>
</tr>
<tr>
<td>3. GOT</td>
<td>0.01 mL (10 µL)</td>
<td>0.01 mL (10 µL)</td>
<td>0.01 mL (10 µL)</td>
</tr>
<tr>
<td>Sample or Standard</td>
<td>0.10 mL (100 µL)</td>
<td>0.10 mL (100 µL)</td>
<td>0.10 mL (100 µL)</td>
</tr>
</tbody>
</table>

b. Mix well by gentle inversion and read absorbances, A1, after 3 minutes.

c. Pipette the following reagent into the cuvettes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDH</td>
<td>0.01 mL (10µL)</td>
</tr>
</tbody>
</table>

4. MDH

| 4. MDH | 0.01 mL (10µL) | 0.01 mL (10µL) | 0.01 mL (10µL) |

d. Mix well by gentle inversion and read absorbances, A2, after 10 minutes.

CALCULATIONS*
1. Calculate the Net Absorbance for the Blank, Sample and Standard:

   \[ \text{Net Absorbance, } A_N = A_2 - A_1 \]

2. Calculate the Corrected Absorbance by subtracting the Net Absorbance for the Blank from the Net Absorbance for the Sample.

   \[ \text{Sample Corrected Absorbance, } A_C = A_N - \text{Blank } A_N \]

3. Do the same for the Standard by substituting the Standard absorbances in place of the Sample absorbances.

4. Calculate the L-Malic acid concentration as follows:

   \[ \text{Malic Acid Concentration (g/L)} = A_C \times 0.4725 \times \text{Dilution Factor} \]

5. Precision (where x is the malic acid concentration in the sample in g/l):

   \[ \text{Repeatability } r = 0.03 + 0.034x \]
   \[ \text{Reproducibility } R = 0.05 + 0.071x \]


REFERENCES