UV assay for the determination of D-malic acid in foodstuffs and other sample materials
Test combination for 50 determinations

For *in vitro* use only
Store between 2 - 8 °C

1. Test principle

D-malic acid (D-malate) is oxidized by nicotinamide-adenine-dinucleotide (NAD) in the presence of D-malate-dehydrogenase (D-MDH) to oxaloacetate, which is immediately converted by this enzyme into pyruvate and carbon dioxide (CO_2).

D-Malate + NAD^+ —D-MDH→ pyruvate + CO_2 + NADH + H^+

The amount of NADH formed is equivalent to the amount of D-malic acid. NADH can be measured on the basis of its absorption at 340 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, D-MDH
- Reagent 2: 2 x 12.5 mL with buffer, NAD^+

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Crush and homogenize solid or semi-solid samples and extract with water; filtrate or centrifuge, or use Carrez clarification if necessary.
- Determination of D-malic acid in wine and grape must: add 125 mg calcium hydroxide and 5 mL ethanol (approx. 98 %) to 25 mL wine, stir for 2 min and adjust to pH 7 - 8 with potassium hydroxide solution (1 M) if necessary. Transfer with bidest. water into a 50 mL volumetric flask, fill up to the mark and mix. Then filter through a folded filter and use clear, colorless or slightly colored filtrate for the test. Strongly colored filtrates and possibly filtrates that show a creep reaction must be decolorized. In this case, proceed as follows: add 2 g moist PVPP to 10 mL filtrate, stir for 2 min, filter through a folded filter and use this filtrate for the test.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 14 - 500 mg/L

	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 25 min at 20 - 37 °C and read absorbance A_2.		

The reagent blank value must be determined once for each run and subtracted from each sample result.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of D-malic acid

$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

$$\mathbf{C_{D\text{-malic acid}}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.5534 \times \Delta A}$$

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 134.09
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

5.2. Calculation of solid samples

$$\text{Content}_{\text{D-malic acid}}\ [g/100\ g] = \frac{C_{\text{D-malic acid}}\ [g/L\ \text{sample solution}]}{\text{weight}_{sample}\ \text{in g/L sample solution}} \times 100$$

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of Enzytec™ *Liquid* Multi-Acid Standard low (E8460).
The recovery of Enzytec™ *Liquid* Multi-Acid Standard low and other aqueous control solutions should be within 100 ± 5 %.

6. Performance data

6.1. Specificity & side activities

The test is specific for D-malic acid.
L-Tartaric acid is side-active and leads to a creeping reaction. For this reason, it must be removed before the measurement as described in chapter 3 "Sample preparation". A remaining amount of L-tartaric acid after precipitation can lead to a small creep reaction, which can, however, be taken into account by calculated extrapolation.

6.2. Interferences

α-Ketoglutaric acid and sulfite do not interfere at a concentration up to 0.5 g/L. Meso-tartaric acid does not interfere at a concentration up to 0.2 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 500 mg/L D-malic acid (sample volume of 100 µL). For a sample volume of 1000 µL, the recommended measuring range is between 1.2 and 50 mg/L.
The limit of detection (LoD) was determined according to method DIN 32645:2008-11, using buffered aqueous solutions. This results in an LoD of 3.7 mg/L for a sample volume of 100 µL or 0.38 mg/L for a sample volume of 1000 µL.
The limit of quantification (LoQ) was determined by precision profile and is 14 mg/L for a sample volume of 100 µL and 1.2 mg/L for a sample volume of 1000 µL.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.37 mg/L. Based on ΔA = 0.010, an LoQ of 0.74 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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