



Enzymatic kit for determination of L-malic acid

Principle:



The determination is based on the formation of NADH measured by the increase in light absorbance at 340 nm

Kit for 45 / 135 analysis includes

Bottle	Composition	Quantity
A.	Buffer	100 ml
B.	Diluent	13 ml
C.	Enzymes	600 µl
STD.	Standard 3 g/l	2 ml

Reagent preparation:

R1: is **Bottle A** and it's ready to use.

R2: preparation for 15 analysis with macro cuvettes (or 45 analysis with semi-micro cuvettes)

Gently mix the **Bottle C**. Pipette 200 µl of **Bottle C** and combine with 4300 µl of **Bottle B**.

Stability of R2: 3 months if stored at 2-8 °C.

Sample preparation:

If necessary, filtrate or centrifuge the sample in order to eliminate the particles in suspension.

Performances:

This test has been developed to determine the concentration of L-malic acid in a measuring range from 0 to 3 g/l.

If the expected values are higher than 3 g/l, samples should be diluted with distilled water and the results must be multiplied by the dilution factor.

Storage instructions and reagent stability:

The reagents are stable up to the expiry date, if stored at 2 to 8°C. Contamination should be avoided. Do not freeze the reagents!

Warnings and precautions:

Do not swallow the reagents. Avoid contact with the skin and mucous membranes.

Take the necessary precautions for the use of laboratory reagents.

Sample analysis

	Blank	Standard	Sample
R1	2200 µl	2200 µl	2200 µl
Water	30 µl		
Standard		30 µl	
Sample			30 µl
Mix and read	DO1 blank	DO1 standard	DO1 sample
R2	300 µl	300 µl	300 µl
Mix wait 15 min and read	DO2 blank	DO2 standard	DO2 sample

Wavelength: 340 nm
Cuvette: 1 cm path (plastic or glass)
Temperature: 20 - 37°C
Zero: against water or air

Calculations:

$$\Delta\text{DO sample} = (\text{DO2-DO1}) \text{ sample} - (\text{DO2-DO1}) \text{ blank}$$

$$\Delta\text{DO standard} = (\text{DO2-DO1}) \text{ standard} - (\text{DO2-DO1}) \text{ blank}$$

$$C \text{ sample (g/l)} = C \text{ standard} \times \frac{\Delta\text{DO sample}}{\Delta\text{DO standard}}$$

Dilution factor of the sample has to be considered in the calculation

It's possible to perform 135 analysis if working with semi-micro cuvettes and applying the volumes below:
R1= 730 µl / Sample = 10 µl / R2= 100 µl