

UNITAB™ REAGENT

L-MALIC ACID

Enzymatic UV -Method

Product # LMA-60 (30 Tests)

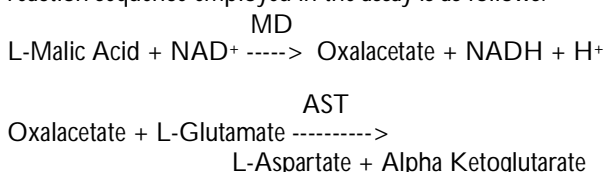
LMA-150 (75 Tests) LMA-500 (250 Tests)

INTENDED USE

Unitech Scientific L-Malic Acid Reagent Tablets are intended for the determination of L-malic acid in wine, juice and other liquid samples.

METHODOLOGY & CHEMICAL PRINCIPLES

This L-malic acid method is based on the method of Mollering.¹ L-Malic Acid Reagent Tablets are optimized to conform to IFU-Analysis Nr. 21-1964.² The enzymatic reaction sequence employed in the assay is as follows:



The primary dehydrogenase reaction is coupled with an aminotransfer reaction. Malate dehydrogenase (MD) catalyzes the oxidation of L-malic acid to oxalacetate with the concomitant reduction of nicotinamide adenine dinucleotide (NAD). The increase in absorbance at 340 nm due to NADH formation is directly proportional to the concentration of L-malic acid in the sample. Removal of oxaloacetate from the reaction system shifts the equilibrium to favor oxidation of malic acid.

REAGENTS

Active ingredients are:	Concentration as Formulated	Quantity/Kit		
		30T	75T	250T
<u>L-Malic Acid Rgt. Tablets</u>		12	30	100
L-Glutamic Acid	90 mM			
NAD	5.3 mM			
<u>Malic Acid Buffer</u>		60	150	500mL
Diethanolamine Buffer	0.5M			
Magnesium	1 mM			
<u>Trigger Enzyme</u>		1.3	3.5	11mL
L-Malate Dehydrogenase	2.4 kU/L			
Aspartate Transaminase	2 kU/L			
Ammonium Sulfate	2.5 M			
<u>L-Malic Acid Standard</u>	0.2 g/L	1	1	5mL

STORAGE & REAGENT PREPARATION

Components are stable until the labeled expiration date when stored in their original container at 2-8°C; store tablets tightly sealed with the desiccant pack provided. Trigger Enzyme, Buffer and Standard are ready to use and require no reconstitution.

Working Reagent (WRgt): Calculate the volume of WRgt required for all samples and standards in the assay. Dissolve each Reagent Tablet in 5 mL Malic Acid Buffer using clean glassware. Mix by gentle inversion. (For high sensitivity assays, refer to NOTES.)

Reconstituted reagent is stable for 4 hours at 2-8°C.

Discard any turbid reagent or reagent having an absorbance greater than 0.7 at 340 nm read against deionized water (DI) blank.

PROCEDURE

System requirements: Wavelength 340 nm, absorbance range 0-2A, pathlength 1.0 cm. For alternative samples volumes (SV), wavelengths, etc. refer to NOTES.

Pipet into Cuvettes	Reagent Blank Cuvette	Sample or Standard Cuvettes
Sample		50 µL
DI water	50 µL	
Working Reagent	2 mL	2 mL
Mix cuvettes and wait 3 minutes. Zero spectrophotometer with Reagent Blank. Read A _{INITIAL} (Initial absorbance).		
Trigger Enzyme	35 uL (1 drop)	35 uL (1 drop)
Mix and wait 10-20 min. Read A _{FINAL} (Final absorbance).		

- Label one cuvette for each sample, standard and blank.
- Prepare sufficient WRgt. Allow WRgt to reach room temperature prior to assay.
- Pipet standard, samples, and water into cuvettes as shown in the Table above using calibrated pipets.
- Dispense WRgt into each cuvette, mix and wait 3 minutes. Zero spectrophotometer with the Reagent Blank cuvette. Read the initial absorbance (A_{INITIAL}) values.
- Gently mix the Trigger Enzyme by inversion and dispense, as shown above. Mix each cuvette, wait 10 -20 minutes and read the final absorbance (A_{FINAL}).

CALCULATIONS

- Calculate the Delta A = A_{FINAL} - A_{INITIAL} for each cuvette.
- If the Delta A for the Reagent Blank is significant, subtract it from each sample and standard Delta A.:
Net A = Delta A_{SAMPLE} - Delta A_{BLANK}
- Samples with Delta A values less than 0.05 should be reassayed with a larger sample volume or less dilute sample.
- Select one of the following calculation methods:
 - Extinction Coefficient (Use standard to verify recovery.)

$$\text{L-Malic Acid (g/L)} = \frac{\text{Net A} \times \text{MW} \times \text{TV} \times \text{df}}{(\epsilon)(P)(1000 \text{ mg/g})(\text{SV})}$$

