UNITECH SCIENTIFIC CALIFORNIA, USA

UNITAB™ REAGENT

L-MALIC ACID

Now linear to **1.5 G/L**

With alternate procedure to 4.0 G/L

Enzymatic UV -Method

3-2016 Revision
Procedure update, see below

Product #:
LMA 60 (30 Tests)
LMA 150 (75 Tests)
LMA 500 (250 Tests)

INTENDED USE

L-Malic Acid UniTAB™ REAGENT is intended for measuring L-Malic Acid concentrations in wine. This reagent is linear to **1.5 G/L** without diluting wine. (For linearity to 4 G/L, refer to APPENDIX.)

KIT CONTENTS:	30T	75T	250T
L-Malic UniTAB Rgt Tablet	12	30	100
Trigger Enzyme	1.3 mL	3.3 mL	2x5.5mL
L-Malic UniTAB Buffer	60 mL	150 mL	500 mL
L-Malic Acid Std., 0.2 G/L	1 mL	1 mL	5 mL

* When testing samples nearing completion of M-L fermentation, use 0.2G/L Standard. Otherwise, to optimize accuracy for samples above 1G/L, substitute the 0.8 G/L Standard, or use a 5-Level L-Malic Standard kit, available from Unitech Scientific.

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 340 nm absorbance over a 0-2 A range with a 1 cm lightpath.

SAMPLES

Centrifuge or filter juice, must or fermentation samples. Decolorize dark red wines (e.g. 0.1 G PVPP Powder/10mL, then filter), or use the 20uL Sample Volume procedure (see Page 2 – "PROCEDURE & CALCULATIONS to 4 G/L.")

REAGENTS

Kit contents are ready to use; they are stable through the labeled expiration date when stored at 2-8 °C. Store tablets tightly sealed with the desiccant pack provided.

ASSAY PREPARATION

Working Reagent, manual*: Prepare the required volume of Working Reagent just prior to testing, based on the number of cuvettes (blank, standard[s], and wine samples) in your assay. Dissolve each Reagent Tablet in 5 mL "Malic UniTAB Buffer" using clean glassware. Mix by gentle inversion.
Working reagent is stable for 4-hrs refrigerated; let reagents reach room temperature prior to beginning assay.
* To prepare ChemWell for Wine™ Working Reagent, refer to APPENDIX AUTOMATED TESTING.

TESTING PROCEDURE, to 1.5G/L without dilution.

1. Pipet water into the Reagent Blank cuvette; pipet standards and samples into respective cuvettes.
2. Pipet Working into cuvettes.
3. Zero spectrophotometer with Reagent Blank.
4. Wait 3 minutes and read initial absorbance (A_1) for Standard and Samples.
5. Gently mix the Trigger Enzyme by inversion , add 1drop to each cuvette, mix and wait 15-20 minutes; read final absorbance (A_2).

Pipet into	Reagent	Sample or

Cuvettes	Blank Cuvette	Standard Cuvettes
Sample		50 µL
DI water	50 µL	
Working Rgt	2 mL	2 mL
Mix and wait 3 minutes. Zero spectrophotometer with Reagent Blank. Read $A_{INITIAL}$ (Initial absorbance)		
Trigger Enzyme	40 uL (1 drop)	40 uL (1 drop)
Mix and wait 15-20 min. Read A_{FINAL} (Final absorbance).		

This procedure test range is **0.03 to 1.5 G/L.** If result is out of range high, dilute, retest, and multiply this result by the dilution factor. Alternatively, for Testing Procedure linearity to 4 G/L (without dilution), refer to APPENDIX.

CALCULATIONS

1. Our online "Flex Calculator™-LMA" spreadsheet is available for download at *http://unitechscientific.com/calculators.htm*. G/L values will be calculated automatically.
2. Manual Calculation:

Calculate ΔA values and G/L as follows for each cuvette:

$$\Delta A = A_{FINAL} - A_{INITIAL}$$

Subtract the ΔA of the Reagent Blank from each sample and standard the ΔA:

$$\text{Net A} = \Delta A_{SAMPLE} - \Delta A_{BLANK}$$

Calculate L-Malic Concentration (based on 0.2 **G/L** Malic Standard); for wine diluted prior to assay, multiply by dilution factor (d.f.)

$$\text{L-Malic G/L} = \frac{\text{Net A}_{SAMPLE} \times (0.2) \times (\text{d.f.})}{\text{Net A}_{STANDARD}}$$

QUALITY CONTROL

It is recommended to monitor assay performance with a check wine (or standard) in each assay.

If using the Alternate Calculations (refer to Appendix), include the standard to monitor reaction completion and assess assay performance. Performance is acceptable if result of standard is within 15% of labeled value.

Factors that may affect the performance of this test include instrument function, temperature, glassware cleanliness, and pipetting accuracy (use calibrated micropipettors.)

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APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

a. Extinction Coefficient (results based on factor; use standard to verify recovery.)

L-Malic Acid (G/L) = 0.901 X Net A

Factor is derived as follows:

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

$$= \frac{\text{Net A x 134.09 x 2.09}}{6.22 \times 1 \times 1000 \times 0.05}$$

Where:

MW =134.09 g/mole

TV = 2.09 mL total reaction volume

SV = 0.05 mL sample volume

ε (absorptivity of NAD) = 6.22 @ 334-340nm

P = 1 cm light path

df = dilution factor (undiluted = 1)

b. Multi-point standard curve Sample concentrations are calculated from the best-fit standard curve. Standard sets available from Unitech Scientific LLC.

TESTING PROCEDURE & CALCULATIONS to 4 G/L

Prepare Working Reagent and dispense reagents as described on Page 1 - except use:

- **20uL Sample Volume** as shown in the table below
- Calculate using:
  - **Standards** (either 0.2, 0.8G/L, or multipoint standard curve), refer to Page 1 Or
  - **Extinction Coefficient** adjusted for 20uL SV:
    L-Malic Acid (G/L)= Net A X 2.220

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		20µL
DI water	20µL	
Working Reagent	2 mL	2 mL
Mix cuvettes and incubate 3 minutes Zero spectrophotometer with Reagent Blank Read $A_{INITIAL}$ (Initial Absorbance)		
Trigger Enzyme	40 uL (1 drop)	40 uL (1 drop)
Mix and incubate 15-20 min. Read A_{FINAL} (Final ABS)		

This procedure test range is 0.08 to 4 G/L. If result is out of range high, dilute, retest, and multiply this result by the dilution factor.

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:

$$\text{L-Malic Acid} + NAD^+ \xrightarrow{\text{MDH}} \text{Oxaloacetate} + NADH + H^+$$

$$\text{Oxaloacetate} + \text{L-Glutamate} \xrightarrow{\text{AST}} \text{L-Aspartate} + \text{Alpha Ketoglutarate}$$

The primary dehydrogenase reaction is coupled with an aminotransfer reaction. Malate dehydrogenase (MD) catalyzes the oxidation of L-malic acid to oxaloacetate 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.

SIGNIFICANCE OF MEASUREMENTS

Free L-Malic Acid is of interest in winemaking and is measured by this method. L-Malic concentration drops from 8 to perhaps 1 g/L in grape must as the ripening process proceeds. Up to 30% of the malic acid may be consumed by yeast fermentation. A secondary fermentation is typical in wine; L-malic acid is converted to L-lactic acid and carbon dioxide by lactic bacteria. Mali-lactic fermentation can be prevented by filtration and increased sulfite.

AUTOMATED TESTING

'ChemWell for Wine™' & 'CW-T for Wine™'

Prepare sufficient L-Malic Working Reagent; the volumes required / # of tests is shown below:

	16T	40T	65T	105T
Tablets	1	2	3	5
DI Water	5mL	10mL	15mL	25mL

(# of Tests accounts for Reagent Bottle dead volume)

Placed the Working Reagent and Trigger Enzyme in CW reagent rack. The testing range for this protocol is up to 1.5 G/L.

Note: Unitech offers both LMA High Sensitivity & Extended Range protocols. Select the appropriate test protocol; contact Unitech Scientific Technical Support for guidance.

CALCULATIONS:

'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range.

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