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

4-2016 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
L-Malic UniTAB Buffer	60 mL	150 mL	500 mL
Trigger Enzyme	1.3 mL	3.3 mL	2x5.5mL
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 turbid samples, such as juice, must or fermentation samples.

Decolorizing Guidelines: Decolorizing is not required for red wines nearing ML completion. When results on red wine samples exceed **0.5 G/L**, decolorize the sample and retest.

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*: Dissolve each Reagent Tablet in 5 mL "Malic UniTAB Buffer" using clean glassware. Mix by gentle inversion. 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.

* For required volumes of ChemWell for Wine™ Working Reagent, refer to APPENDIX AUTOMATED TESTING.

Working reagent is stable for 4-hrs refrigerated; let reagents reach room temperature prior to beginning assay.

TESTING PROCEDURE to 1.5G/L

(Refer to APPENDIX for the alternative testing procedure providing linearity to 4 G/L without dilution)

Pipet each solution (#1-4) into the cuvettes, as shown:

		Blank	Standard	Sample(s)
1.	**D.I. Water**	**50µL**		
2.	**Standard/Sample**		**50µL**	**50µL**
3.	**Working Reagent**	**2.0mL***	**2.0mL**	**2.0mL**

Mix and incubate 3 minutes.

Zero spectrophotometer (340 nm) with Reagent Blank

Read A_0 (Initial ABS) **Note: 2.0mL = 2000µL*

Add (One Drop) Trigger Enzyme

4.	**Trigger**	**40µL**	**40µL**	**40µL**

Mix and incubate 20 minutes, Read A_{FINAL} (Final ABS).

If any test result is over-range, dilute the sample with deionized (or distilled) water; re-assay & multiply this test result by the dilution factor.

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.)

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

QUALITY CONTROL

We recommend monitoring assay performance with a check wine (or standard) in each assay. If calculating by Extinction Coefficient (refer to Appendix), include the standard to monitor 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; compare standard result with label concentration to verify assay performance.)

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 to 4 G/L

Prepare Working Reagent and dispense reagents as described on Page 1 - except use **20uL Sample Volume** as show below:

Pipet the following volumes (#1-4) into the cuvettes:

	Blank	Standard	Sample(s)
1. **D.I. Water**	20µL		
2. **Standard/Sample**		20µL	20µL
3. **Working Reagent**	2.0mL*	2.0mL	2.0mL

Mix cuvettes, incubate 3 minutes.

Zero spectrophotometer (340 nm) with Reagent Blank

Read $A_{INITIAL}$ (Initial ABS)

**Note: 2.0mL = 2000µL*

Add (One Drop) Trigger Enzyme

4. **Trigger Enzyme**	40µL	40µL	40µL

Mix cuvettes, incubate 15-20 minutes, Read A_{FINAL} (Final ABS).

CALCULATIONS to 4 G/L 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

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

This method measures Free L-Malic Acid; the concentration in grapes drops during ripening from 8 to as low as 1 g/L. In grape must, up to 30% of the malic acid may be consumed by yeast fermentation. A secondary fermentation by lactic bacteria is typical in wine; L-malic acid is converted to L-lactic acid and carbon dioxide. Wine may be considered "MLF stable" at malic levels less than 0.3 G/L. Mali-lactic fermentation can be prevented by filtration and increased sulfite.

AUTOMATED TESTING

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

CW Protocols: Unitech Scientific provides Malic Acid UniTAB protocols for both LMA High Sensitivity & Extended Range. Select the appropriate test protocol. Contact Unitech Scientific for technical support.

Decolorizing red wine is typically not required.

Working Reagent: Prepare the required volume of Working Reagent shown below:

	16T	40T	65T	105T
Tablets	1	2	3	5
Malic Buffer	5mL	10mL	15mL	25mL

(# of Tests accounts for Reagent Bottle dead volume)

Placed the Working Reagent and Trigger Enzyme in CW reagent rack.

Unitech provides a 5-standard Kit (STD MA); contact Unitech Scientific.

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