

L-MALIC ACID Now linear to **1.5 G/L**
 With alternate procedure to 4.0 G/L

Product #:
LMA F60 (30 Tests)
LMA F150 (75 Tests)
LMA F500 (250 Tests)

3-2016 Revision
 Procedure update, see below

Enzymatic UV-Method

INTENDED USE

L-Malic Acid UniFLEX™ 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
Mali-Lactic Buffer	20mL	50mL	170mL
NAD Solution	6 mL	15mL	51mL
GOT Suspension	0.4mL	1mL	3x1mL
MDH Suspension	0.4mL	1mL	3x1mL
L-Malic Acid 0.2 G/L Std *	1mL	5mL	5mL

* 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 a 0-2 A range of 340 nm absorbance readings with a 1 cm lightpath.

SAMPLES

Centrifuge or filter turbid samples – such as 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 (refer to P2 – “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.

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.

	Per test
Mali-Lactic_Buffer	0.67mL
NAD Solution	0.2mL
GOT Suspension	0.010mL
Deionized Water	1.33mL
WRgt (Approx.Total)	2mL

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 Reagent into cuvettes and incubate as shown in the table.
3. Wait 3 minutes, zero spectrophotometer with Reagent Blank and read initial absorbance (A_{INITIAL}).
4. Gently mix MDH Suspension by inversion and pipet into cuvettes, mix.
5. The Incubate as specified in Table, read Final Absorbance.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		50µL
DI water	50µL	
Working Reagent	2 mL	2 mL
Mix cuvettes and incubate 3 minutes Zero spectrophotometer with Reagent Blank Read A _{INITIAL} (Initial Absorbance)		
MDH Suspension	10 uL	10 uL
Mix and incubate 15-20 min. Read A _{FINAL} (Final ABS)		

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 at <http://unitechscientific.com/calculators.htm> is available for download. G/L values will be calculated automatically.

2. **Manual Calculation:**

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

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

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

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

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

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

QUALITY CONTROL

We recommend you monitor assay performance with a check wine (or standard) in each assay. If calculating by Extinction Coefficient (refer to Appendix), include 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 micro-pipettors.)

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

- a. **Extinction Coefficient** (results based on factor; compare standard result with known value to verify recovery.)

$$\text{L-Malic Acid (G/L)} = \text{Net A} \times 0.888$$

Factor is derived as follows:

$$\begin{aligned} \text{Malic Acid (G/L)} &= \frac{\text{Net A} \times \text{MW} \times \text{T.V.} \times \text{df}}{(\epsilon)(P)(1000\text{mG/G})(\text{SV})} \\ &= \frac{\text{Net A} \times 134.09 \times 2.06}{6.22 \times 1 \times 1000 \times 0.05} \end{aligned}$$

Where:

$$\text{Net A} = \Delta A - \Delta A_{\text{Blank}}$$

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

MW = 134.09G/mole for malic acid

*TV = 2.06 mL total reaction volume

*SV = 0.05 mL sample volume (See Notes)

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

P = 1 cm light path

df = dilution factor (undiluted = 1)

Adjust calculations if alternate SV and TV are used. Sample volume inaccuracy will affect results with the extinction coefficient calculation method; use calibrated micropipettes.

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

TESTING PROCEDURE & CALCULATIONS to 4 G/L

Prepare Working Reagent and dispense reagents as described on Page 1. **Pipet 20uL DI Water, Standard, and Sample** 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:

$$\text{L-Malic Acid (G/L)} = \text{Net A} \times 2.188$$

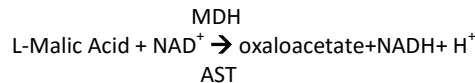
Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Standard(s), Samples		20µL
DI water	20µL	
Working Reagent	2 mL	2 mL
Mix cuvettes and incubate 3 minutes Zero spectrophotometer with Reagent Blank Read A_{INITIAL} (Initial Absorbance)		
MDH Suspension	10 uL	10 uL
Mix and incubate 15-20 min. 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

The assay methodology of this reagent is based on the method of Mollering.¹ L-Malic Acid FLEX-Reagents are optimized to conform to

IFU-Analysis Nr. 21-1964.² The enzymatic reaction sequence employed in the assay is as follows:



Oxaloacetate + L-Glutamate \rightarrow L-Aspartate + Alpha Ketoglutarate

The primary dehydrogenase reaction is coupled with an amino transfer reaction. Malate Dehydrogenase (MDH) catalyzes the oxidation of L-malic acid to oxaloacetate with the concomitant reduction of nicotinamide adenine dinucleotide (NAD). The increase in absorbance at 340nm 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 WineTM' & 'CW-T for WineTM'

L-Malic analysis uses the following CW-Working Reagent; **do NOT add NAD Solution** to CW Working-Reagent. The volumes required / # of tests is shown below:

	25T	40T	75T
Mali-Lactic Buffer	2mL	3mL	5mL
GOT Suspension	0.030mL	0.045mL	0.080mL
Deionized Water	4.0mL	6.0mL	10mL
Approx.Total	6mL	9mL	15mL

(# of Tests accounts for Reagent Bottle dead volume)

Unitech provides a 5-standard ("STD MA High") set ; contact Unitech Technical Service for more information.

Place Working Reagent, NAD Solution (independently dispensed by CW) and MDH Enzyme in CW reagent rack.

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.

TRADEMARKS:

"ChemWell for Wine", "Flex Calculator", "FLEX Reagent" are Trademarks of Unitech Scientific LLC

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