

### L-MALIC ACID **MALIC DRY RANGE** - Improved New option, ChemWell Auto-Prep tests (refer to NOTE)

**LMA F60 (30 Tests)**  
**LMA F150 (75 Tests)**  
**LMA F500 (250 Tests)**

#### Enzymatic UV

#### INTENDED USE

**L-Malic Acid UniFLEX™ REAGENT** is intended for measuring L-Malic Acid concentrations in wine up to 1.5G/L - for wines undergoing ML fermentation.

Extended Range (LMA CW reagent kit) & procedure is available for testing juice & wine with Malic concentrations up to 4 G/L without dilution or decolorizing.) Contact Unitech Scientific for details.

#### KIT CONTENTS

	<u>30T</u>	<u>75T</u>	<u>250T</u>
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

\* A 5-Level L-Malic Standard kit is 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.

**Decolorizing Guidelines:** Decolorizing is not required for red wines nearing ML completion, i.e. **0.03 - 0.5 G/L**. Decolorize red wines with higher levels, **0.5 to 1.5 G/L** Malic Acid.

#### 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

Prepare Working Reagent just prior to testing, based on the number of blank, standards and wine samples in your assay.

	<u>Per test (2mL)</u>
Mali-Lactic_Buffer	0.67mL
NAD Solution	0.20mL
GOT Suspension	0.010mL
Deionized Water	<b>1.20mL</b>

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

#### TESTING PROCEDURE

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

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

Mix and incubate 3 minutes. *\*Note: 2.0mL = 2000µL*

Zero spectrophotometer (340 nm) with Reagent Blank

Read A<sub>0</sub> (Initial ABS)

Add MDH Enzyme

4. <b>MDH Solution</b>	<b>10µL</b>	<b>10µL</b>	<b>10µL</b>
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Mix and incubate **20 minutes**, Read A<sub>FINAL</sub> (Final ABS).

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

#### 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; for wine diluted prior to assay, multiply by dilution factor (d.f.)

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

#### 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.)

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

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)

$\epsilon$  = 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.

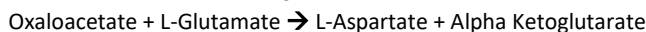
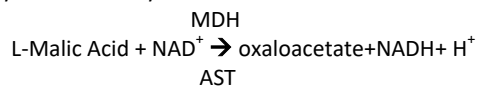
- b. **Multi-point standard curve** Sample concentrations are calculated from the best-fit standard curve. Standard sets are available from Unitech Scientific.

### 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.

### METHODOLOGY & CHEMICAL PRINCIPLES

The assay methodology of this reagent is based on the method of Mollering.<sup>1</sup> L-Malic Acid FLEX-Reagents are optimized to conform to IFU-Analysis Nr. 21-1964.<sup>2</sup> The enzymatic reaction sequence employed in the assay is as follows:



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.

### AUTOMATED TESTING - ChemWell for Wine, CW-T for Wine

**CW Malic Protocols:** Unitech Scientific has recently **optimized protocols** for both **LMA Dry Range & MLA Extended Range**; decolorizing red wine is typically not required. For best results, contact Unitech Scientific technical support to assure you have the latest procedure.

**Working Reagent** for Automated Testing is stable for 2-days refrigerated; prepare per instructions below. **Consider Unitech's optional "AP" tests - Working Reagent preparation is automated.** Refer to details: **(Optional) AP Auto-Prep NOTE** below.

Prepare the Working Reagent as shown in the table; **(NAD Solution is not added to Working Reagent):**

	<b>20T</b>	<b>45T</b>	<b>90T</b>
<b>Mali-Lactic Buffer</b>	1.5mL	3mL	6mL
<b>GOT Suspension</b>	0.025mL	0.05mL	0.100mL
<b>Deionized Water</b>	2.5 mL	5.0mL	10mL
<b>Approx.Total</b>	4mL	8mL	16mL

(# of Tests accounts for Reagent Bottle dead volume)

Place Working Reagent, NAD Solution and MDH Enzyme in ChemWell reagent rack, according to software prompts.

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

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\* **NOTE: (Optional) AP Auto-Prep ChemWell & CW-T for Wine tests.** Simply place each kit component directly in Reagent Rack. **With AP protocols, your CW will:**

- ✓ Automatically prepare Working Reagent in each cuvette
- ✓ Optimize your reagent usage & improve lab work flow.

With Reagent Cooling and **AP protocols**, UniFLEX reagents are stable in your ChemWell reagent rack for months.

Contact Unitech Scientific Tech Service for your new **AP protocol**

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### TRADEMARKS:

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

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