UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

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 in wine samples nearing ML completion; it provides very low detection (down to **0.03G/L**) and up to 1.5G/L.

Also Available from Unitech Scientific: Extended Range **LMA CW** reagent kit & procedure for testing juice & wine with Malic concentrations up to 4 G/L without dilution. There is no need to decolorize red wines up to 2G/L Malic concentration with this procedure; it offers sensitive detection (down to **0.08G/L.**) Contact Unitech Scientific for details.

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

* 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. 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.

ASSAY PREPARATION

Working Reagent

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

	Per test (2mL)
Mali-Lactic_Buffer	0.67mL
NAD Solution	0.20mL
GOT Suspension	0.010mL
Deionized Water	1.20mL

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:

	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. **Note: 2.0mL = 2000µL*

Zero spectrophotometer (340 nm) with Reagent Blank

Read A_0 (Initial ABS)

Add MDH Enzyme

4. **MDH Solution**	**10µL**	**10µL**	**10µL**

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

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:

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

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

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

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

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

If any result >1.5G/L, dilute sample with deionized water; re-assay & multiply this test result by the dilution factor.

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)= Net A X 0.888

Factor is derived as follows:

$$\text{Malic Acid (G/L)} = \frac{\text{Net A} \times \text{MW} \times \text{T.V.} \times \text{df}}{(\varepsilon)(P)(1000\text{mG/G})(SV)}$$

$$= \frac{\text{Net A} \times 134.09 \times 2.06}{6.22 \times 1 \times 1000 \times 0.05}$$

Where:

Net A = Delta A - Delta A_{Blank}

Delta A = $A_{FINA}L$- $A_{INITIAL}$

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.

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.[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:

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

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

The primary dehydrogenase reaction is coupled with an 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; decolrizing 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):

	20T	**45T**	**90T**
Mali-Lactic Buffer	1.5mL	3mL	6mL
GOT Suspension	0.025mL	0.05mL	0.100mL
Deionized Water	2.5 mL	5.0mL	10mL
Approx.Total	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.

*** 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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