

L-MALIC ACID EXTENDED RANGE manual & CW

Improved High Malic procedure, see updates below
 New option, ChemWell Auto-Prep tests (refer to **NOTE**)

Product #: **LMA CW F60 (30 Tests)**
LMA CW F150 (75 Tests)
LMA CW F500 (250 Tests*)
 * sufficient for 2,000 CW tests

Enzymatic UV

INTENDED USE

L-Malic Acid UniFLEX™ CW-REAGENT is intended for measuring L-Malic Acid concentrations using both manual and ChemWell automated procedures in samples with L-Malic Acid concentrations between **0.08 - 4.0 G/L**

KIT CONTENTS

	30T	75T	250T
Mali-Lactic Buffer	20mL	50mL	170mL
NAD Solution	2 X 6mL	2 X 15mL	2 X 51mL
GOT Suspension	0.4mL	1mL	3x1mL
MDH Suspension	0.4mL	1mL	3x1mL
L-Malic Acid 0.2 G/L Std *	1mL	5mL	5mL

* For samples above 1G/L, substitute Malic Acid **0.8 G/L** Standard; observe the alternate calculation factor, as shown.**
 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 < **2 G/L** Malic Acid concentrations. Consider decolorizing wine samples with higher Malic levels.

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 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	0.60 mL
NAD Solution	0.40 mL
GOT Suspension	0.010 mL
Deionized Water	1.0 mL

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

TESTING PROCEDURE

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

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

Mix and incubate 3 minutes.

Zero spectrophotometer (340 nm) with Reagent Blank

Read A₀ (Initial ABS) * Note: 2.0mL = 2000µL

4. MDH Solution	10µL	10µL	10µL
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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

Calculate L-Malic Concentration

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:

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

Standard Method Calculations:

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

Where d.f. = dilution factor if any.

**Substitute 0.8 if using the 0.8 G/L Standard.

Sample volume inaccuracy will affect results with this extinction coefficient calculation method; use calibrated micropipettes.

QUALITY CONTROL

Monitor assay performance using a check wine (or standard) in each assay. 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.)

MANUFACTURED BY: **UNITECH SCIENTIFIC**
 12026 Centralia Road Suite H, Hawaiian Gardens, CA 90716
 Tel: 562-924-5150 Fax: 562-809-3140

<http://unitechscientific.com>

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

a. Extinction Coefficient Method Calculation:

$$\text{L-Malic Acid (G/L)} = \text{Net A} \times 2.188 \\ (\text{adjusted for 20}\mu\text{L Sample Volume}^*)$$

Factor is derived as follows:

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

MW = 134.09G/mole

TV = 2.03 mL total reaction volume

SV = 0.02 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.

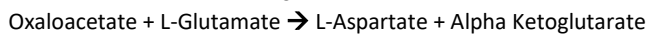
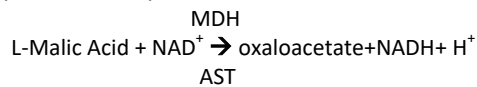
b. Multi-point Standards and best-fit standard curve calculation methods are available from Unitech Scientific.

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. Malic-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:



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 High Sensitivity & Extended Range; decolorizing red wine is typically not required. For best results, contact Unitech Scientific for technical support to update your files.

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

TRADEMARKS:

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

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www.unitechscientific.com