UNITECH SCIENTIFIC CALIFORNIA, U.S.A

FLEX-REAGENT™

L-MALIC ACID *ChemWell for Wine Extended Range*

*Linear Range **0.03-4.0 G/L**, Procedural Changes*

Enzymatic UV-Method

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

INTENDED USE

L-Malic Acid UniFLEX™ CW Extended Range REAGENT is primarily intended for ChemWell automated measurement of L-Malic Acid concentrations in wine; a manual procedure is included. This procedure is linear between 0.03 - 4.0 G/L.

KIT CONTENTS

	30T	75T	250T
Mali-Lactic Buffer	20mL	50mL	170mL
Mali-Lactic NAD	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

* To optimize calibration for samples above 1G/L, substitute Malic Acid 0.8 G/L Standard, available from Unitech Scientific. Refer to APPENDIX - OPTIONAL calculation.

SYSTEM REQUIREMENT

Spectrophotometer should be capable of a 0-2 A range of 340 nm absorbance readings with a 1 cm lightpath.

SAMPLES

If wine samples are visually clear, no sample pretreatment is needed. Filter or centrifuge turbid samples, e.g. juice, must or fermentation samples.

REAGENTS

Kit contents are ready to use are stable through the labeled expiration date when stored at 2-8 °C.

ASSAY PREPARATION

Working Reagent, manual:
(For 'ChemWell-for-Wine'™ test details, see Appendix)
Prepare Working Reagent just prior to testing, based on the number of blank, standard and wine samples in your assay.

	1T
Mali-Lactic_Buffer	0.67mL
GOT Suspension	0.010mL
Deionized Water	1.33mL
WRgt (Approx.Total)	2mL

Note that in BOTH manual and automated procedures, NAD Solution is added directly to each cuvette. **Without NAD,** Working reagent is stable for 5-days refrigerated; allow it to reach room temperature prior to assay.

TESTING PROCEDURE (0.03 - 4.0G/L without dilution)

1. Pipet water into the Reagent Blank cuvette; pipet standards and samples into respective cuvettes.
2. Pipet Working Reagent into cuvettes, followed by NAD Solution, and incubate as shown in the table.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		50µL
DI water	50µL	
NAD Solution	450 µL	450 µL
Working Reagent	1.60 mL	1.60 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 8-10 min. Read A_{FINAL} (Final ABS)		

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. Incubate as specified in Table, read Final Absorbance.

Testing range 0.03 - 4.0 G/L, if an over-range result is obtained, dilute sample and retest. Multiply this test result by the dilution factor.

*NOTE: Also available from Unitech, improved LMA FLEX kit with measuring range is 0.03 - 1.5 G/L, using less NAD Solution: http://unitechscientific.com/us-di/LMA-Flex.pdf

CALCULATIONS

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** Std, provided))

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

QUALITY CONTROL

Test a standard in each assay to calculate wine results. 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.) A 5-Level L-Malic High Standard kit is available from Unitech Scientific.

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS:

a. Standard Calculation - OPTIONAL procedure – To optimize calibration for samples above 1G/L, substitute Malic Acid 0.8 G/L Standard:

Calculate L-Malic Concentration

$$\text{L-Malic G/L} = (0.8) \times (\text{d.f.}) \times \frac{\text{Net A}_{\text{SAMPLE}}}{\text{Net A}_{\text{STANDARD}}}$$

b. Extinction Coefficient (results based on factor; compare standard result with known value to verify recovery.)
Malic Acid (G/L)= Net A X 0.910

Factor is derived as follows:

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

$$= \frac{\text{Net A} \times 134.09 \times 2.11}{6.22 \times 1 \times 1000 \times 0.05}$$

$$= \text{Net A} \times 0.910$$

Where:

Net A = Delta A - Delta A_{Blank}

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

MW =134.09G/mole for malic acid

TV = 2.11 mL total reaction volume

SV = 0.05 mL sample volume

ε = absorptivity of NAD
=6.22 @334-340nm; 3.4 @ 365nm

P = 1 cm light path

Recalculate if alternate WRgt & SV are used.

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

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

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.

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 Wine™' & 'CW-T for Wine™'

Prepare Working Reagent as described in ASSAY PREPARATION for manual assays; the number of tests expected for each preparation 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.

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