UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

L-LACTIC ACID_NF Improved Reagent & Procedure

NEW FORMAT *UV-Endpoint Method* for WINE revised Calculations & Automation

LLA-F40_NF (40 Tests)
LLA-F100_NF (100 Tests)
'Tests/kit' assumes **1mL W-Rgt/test**

INTENDED USE

Unitech Scientific L-Lactic Acid FLEX-Reagents™ are intended for the determinations of L-Lactic acid in wine, juice and other liquid samples.

REAGENTS: L-Lactic Acid FLEX-Reagent kits

	40T	100T
Sample Blank Solution (used for automation only)	40mL	100mL
R1 Enzymes: LDH/ALT	40mL	100mL
R2 NAD Solution	4mL	10mL
L-Lactic Acid Std. 0.6 G/L	3mL	3mL

REAGENT PREPARATION & STORAGE

Reagents and standards are liquid and ready to use; in this NEW reagent FORMAT, no reagent preparation is required for manual testing. (Do not mix these reagent components with the original format LLA reagents.) Kit components are stable until labeled expiration date when stored in their original containers at 2-8°C.

SAMPLES

Juice and fermentation samples must be centrifuged or filtered; typically decolorizing samples is not needed.

PROCEDURE (manual method)

System parameters: Wavelength 340 nm, Absorbance Range 0-2A, pathlength 1.0 cm.
Allow reagents to reach room temperature.

1. Pipet water into the Reagent Blank cuvette and pipet standards, controls, samples into cuvettes as shown.
2. Dispense **R1 Enzyme** Solution, mix and incubate at 37°C. Zero spectrophotometer with the Reagent Blank cuvette. Read the initial absorbance (A_1) values.
3. Dispense the **R2 NAD** Solution as shown. Mix each cuvette, incubate at 37°C & read final absorbance (A_2).

Pipette into Cuvettes	Blank Cuvette	Reaction Cuvettes
Std/Sample	-	20µL
DI water	20µL	-
R1 Enzyme	1000uL (=1mL)	1000uL (=1mL)
Mix cuvettes and incubate 2 min. at 37°C Zero spectrophotometer with Reagent Blank Read **A_1 (Initial ABS)**		
R2 NAD	100uL	100uL
Mix and incubate 15-20 min. at 37°C Read **A_2 (Final ABS)**		

CALCULATIONS

1. Calculate the **Net A_1** for Blank, Standard and each sample cuvette (to correct for the R2 Volume):
   Net A_1 = 0.911 X **A_1**
2. Compute L-Lactic Acid Levels, based on the 0.6 G/L standard:
   Lactic Acid G/L =

$$0.6 \times \frac{(A_{2\text{-SAMPLE}} - \text{Net } A_{1\text{-SAMPLE}}) - (A_{2\text{-BLANK}} - \text{Net } A_{1\text{-BLANK}})}{(A_{2\text{-STANDARD}} - \text{Net } A_{1\text{-STANDARD}}) - (A_{2\text{-BLANK}} - \text{Net } A_{1\text{-BLANK}})}$$

For samples > 0.65 G/L, dilute with D.I. Water and re-assay; multiply result by dilution factor; e.g. mix 1 part Wine + 2 parts DI Water, multiply G/L result by dilution factor (=3.)

APPENDIX

Automation Procedure:

Place **R1 Enzyme, R2 NAD Solution**, and **Sample Blank Solution** in reagent rack. Contact Unitech for "ChemWell for Wine" and "CW-T for Wine" assay programing. Analyzer will correct for wine color and calculate G/L.

METHODOLOGY & CHEMICAL PRINCIPLES

L-Lactic Acid is oxidized by NAD to pyruvate by L-LDH and excess pyruvate is removed by ALT enzyme, as follows:

$$\text{L-Lactic Acid} + NAD^+ \xrightarrow{\text{L-LDH}} \text{pyruvate} + NADH$$

$$\text{Pyruvate} + \text{L-Glutamate} \xrightarrow{\text{ALT}} \text{L-Alanine}$$

The increase in absorbance at 340nm due to NADH formation is directly proportional to the concentration of L-Lactic Acid in the sample. Removal of pyruvate from the reaction system shifts the equilibrium to favor oxidation of Lactic Acid.

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