UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

D-LACTIC ACID New Reagent & Procedure

***UV-Endpoint Method* for WINE**

DLA-F40 (40 Tests)

Tests/kit assumes **1mL W-Rgt/test**

INTENDED USE

Unitech Scientific D-Lactic Acid FLEX-Reagents™ are intended for the determinations of D-Lactic acid in wine. D-Lactic Acid is a marker for microbial wine spoilage.

REAGENTS:

	40T
Sample Blank Solution	40mL
Chromogen Diluent	20mL
R1 Chromogen	20mL
R2 Enzymes: D-LDH/GPT	2.05mL
D-Lactic Acid Std. 0.6 G/L	2mL

REAGENT PREPARATION & STORAGE

Kit components are liquid, ready to use, and stable until labeled expiration date when stored at 2-8°C.

Prepare Working Reagent: Mix sufficient **Working-Rgt** Solution, as shown in the example:

	10 Tests	20 Tests
Chromogen Diluent	5mL	10mL
Chromogen	5mL	10mL
R2 Enzyme	0.50mL	1.0mL

Working-Rgt is stable for 3 hours at 2-8°C. Allow reagents to reach room temperature.

SAMPLES

Clarify fermentation and juice samples. It is not necessary to decolorize red wine.

PROCEDURE (manual method)

System parameters: Wavelength 340 nm, Absorbance Range 0-2A, pathlength 1.0 cm.

1. The Zero-Rgt Blank & Zero-Rgt Reaction Cuvette will be used to zero the spec.
   Pipet **DI Water** into each cuvette, as shown in the table.
2. Label two sets of cuvettes: Blank & Reaction Cuvette.
   Pipet each **Standard & Sample** into both (BL & Rx) sets of cuvettes, as shown in the table.
3. Dispense **Sample-BL** & **Working-Rgt** solutions to labelled cuvettes (refer to Table). Mix, incubate at 37°C.
4. Zero the Spec with **Zero-Rgt Blank Cuvette**; read Standard and Sample Blank Cuvettes.
5. Re-zero Spec with **Zero-Rgt Reaction Cuvette;** read Standard and Sample **Reaction cuvettes**.

ADD	Volume/Cuvette			
	Zero-Rgt Cuvettes		Standard &Sample	
	Blank Cuvette	Reaction Cuvette	Blank Cuvette	Reaction Cuvette
DI Water	20µL	20µL	-	-
Standard or Sample			20µL	20µL
Sample Bl Soln	**1000µL**	-	**1000µL**	-
W-Rgt	-	1000µL	-	1000µL
Mix, warm to 37C for 10-minutes Zero Spec & **Read Blank Cuvettes** Zero Spec & **Read Reaction Cuvettes**				
Zero Spec & Read ABS	**A = 0** *"Zero* **Rgt Blank Cuvette"**	**A = 0** *"Zero* **Reaction Cuvette"**	$A_{SAMPLE-BL}$	A_{SAMPLE}

CALCULATIONS (manual method)

Calculate Net ABS values by subtracting corresponding Blank ABS values from Reaction ABS values:

$$\text{Net } A_{STD} = A_{STD} - A_{STD\text{-}BL}$$

$$\text{Net } A_{SAMPLE} = A_{SAMPLE} - A_{SAMPLE\text{-}BL}$$

Calculate D-Lactic concentrations (based on the 0.6 g/L standard):

$$\textbf{Lactic Acid G/L} = 0.6 \times \frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STD}}$$

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.)

Automation Procedure:

Calculate volume required and prepare Working Reagent as shown below; this Working-Rgt is stable for 3 days at 2-8°C.

	20 Tests
Chromogen Diluent	2.5mL
Chromogen	2.5mL

Estimated tests accounts for Reagent Bottle dead volume. Place the Sample Blank Solution (supplied), Working Reagent and R2 Enzyme in the reagent rack. Contact Unitech for Assay programing for "ChemWell for Wine" and "CW-T for Wine". Analyzer will automatically calculate G/L.

APPENDIX
METHODOLOGY & CHEMICAL PRINCIPLES

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

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

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

The increase in absorbance at 340nm due to NADH formation is directly proportional to the concentration of D-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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