

L-LACTIC ACID

Enzymatic UV-Method

Product #: LLA-F60 (30 Tests)

LLA-F150 (75 Tests)

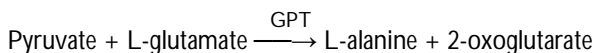
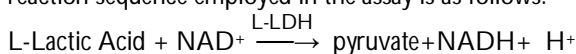
LLA-F500 (250 Tests)

INTENDED USE

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

METHODOLOGY & CHEMICAL PRINCIPLES

The assay methodology of this reagent is based on the method of Gutmann and Wahlefeld.¹ The enzymatic reaction sequence employed in the assay is as follows:



The primary dehydrogenase reaction is coupled with an amino transfer reaction. L-lactate dehydrogenase (L-LDH) catalyzes the oxidation of L-Lactic Acid to pyruvate with the concomitant reduction of nicotinamide adenine dinucleotide (NAD). The increase in absorbance at 340nm due to NADH formation is directly proportional to the lactic acid concentration in the sample. Enzymatic removal of pyruvate from the reaction system by GPT shifts the equilibrium to favor oxidation of lactic acid.

REAGENTS

L-Lactic Acid FLEX-Reagent active ingredients are:

	Concentration as Formulated	Quantity/Kit
1. <u>Mali-Lactic Buffer</u>		75T 250T
Glycylglycine	1.5 M	50mL 170mL
L-Glutamic Acid	100 mM	
Stabilizers, pH 10		
2. <u>NAD</u> a. Powder	55 mM final	
b. NAD Diluent		14mL 47mL
3. <u>GPT Solution</u>	0.9 KU/mL	3.3mL 11mL
4. <u>L-LDH Suspension</u>	2.7 KU/mL	3.3mL 11mL
7. <u>L-Lactic Acid Standard</u>	0.2G/L	2mL 2mL

REAGENT PREPARATION & STORAGE

- Mali-Lactic Buffer, GPT and LDH enzyme suspensions and standards are ready to use; gently mix suspensions by inversion prior to use. Reagent components are stable until labeled expiration date when stored in their original container at 2-8°C.
- Prepare NAD Solution by quantitatively transferring the NAD Diluent provided into the NAD Powder; label dissolution date. NAD Solution is stable for 3 months when stored refrigerated (2-8° C.)

PROCEDURE

- Working Reagent (WRgt): Allow Mali-Lactic Buffer to reach room temperature. Mix both LDH and the GPT suspensions by gentle inversion prior to use.

Precaution: Perspiration contains L-lactic acid. Do not touch pipette tips with fingers.

- Prepare sufficient WRgt for each sample and standard in the assay: Working reagent is stable for 8 hours (2-8° C); discard any turbid working reagent or if 340 nm absorbance is > 0.7 when read against distilled water.

	1 Test	3 Tests	10 Tests	26 Tests
Mali-Lactic_Buffer (Bottle1)	0.67mL	2 mL	6 mL	16 mL
NAD Solution	0.2 mL	0.6 mL	1.8 mL	4.8 mL
GPT Suspension (Bottle3)	0.040mL	0.12 mL	0.36mL	0.960mL
Deionized Water	1.34 mL	4 mL	12 mL	32 mL
WRgt (Approx.Total)	2.2 mL	6.6mL	20 mL	53 mL

- Pipet water into the Reagent Blank cuvette and pipet standards, controls, samples into cuvettes as shown.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample		50µL
DI water	50µL	
Working Reagent	2 mL	2 mL
Mix, incubate 5', read absorbances (A ₁).		
**Mix, incubate 30' and read absorbances (A ₂).		
L-LDH Suspension (#5)	40 uL (1 drop)	40 uL (1 drop)
Mix, incubate 25' and read absorbances (A ₃).		

System parameters: Wavelength 340 nm, Absorbance Range 0-2A, pathlength 1.0 cm. Refer to NOTES for alternatives.

- Dispense WRgt, mix and incubate 5 minutes. Zero spectrophotometer with the Reagent Blank cuvette. Read the ABS₁ values.
- Gently mix the L-LDH Suspension and dispense as shown above. Mix each cuvette, incubate and read the ABS₃ (final absorbance) values.

CALCULATIONS

- Calculate the absorbance difference for the sample:
 $A_2 - A_1 = \Delta \text{ABS}_{\text{L-lactate}}$. If the Reagent Blank (Step 3) ΔABS is significant, subtract the absorbance difference of the blank from that of each sample and standard to correct for reagent-dependant absorbance drift.
- Compute Lactic Acid Levels by one of the following:
 - A single point standard, for example 0.2 G/L.

$$\text{Lactic Acid, G/L} = \text{Conc. Std.} \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}}$$

$$= 0.2 \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}}$$

- b. A multi-point standard curve run with each assay. Sample concentrations are calculated from the best-fit standard curve.
- c. Extinction Coefficient (Use the 0.2 G/L Standard as a control to verify recovery.)

$$\begin{aligned} \text{L-Lactic Acid (G/L)} &= \frac{A_{2-1} \times \text{MW} \times \text{T.V.}}{(\epsilon)(P)(1000\text{mG/G})(\text{SV})} \\ &= \frac{A_{2-1} \times 90.1 \times 2.09}{6.22 \times 1 \times 1000 \times 0.05} \\ &= A_{2-1} \times 0.6055 \end{aligned}$$

Where:

- A_{2-1} = delta Absorbance of sample
 MW = 90.1 G/mole for lactic acid
 *TV = 2.09 mL total reaction volume
 *SV = 0.05 mL sample volume (See Notes)
 ϵ = absorptivity of NAD = 6.22 @334-340nm; 3.4 @ 365nm
 P = 1 cm light path
 * Recalculate if alternate WRgt & SV are used. See Notes.
 Inaccuracy in SV delivery will affect results with this calculation method. Use calibrated micropipettes.

If samples have been diluted during preparation, multiply the results by the dilution factor.

SAMPLES

Test solutions should be clear liquids; cloudy or turbid samples may be centrifuged or filtered; degas samples containing dissolved CO₂ (e.g. by filtration). Red wine typically does not need decolorization and may be assayed directly for free lactic acid.

Significance of Measurements: Lactic acid is found in very low concentration in grapes. Between 0.1 – 0.4 G/L of lactic acid² is typically found in wine. A small proportion of this (typically L-lactic acid) is produced by yeast during primary fermentation, while larger quantities of D-lactic acid may be produced by lactic bacteria metabolism of malic acid during secondary fermentation. Infrequently, and in the presence of high residual sugar and pH, ubiquitous lactic bacteria (typically of *Leuconostoc*, *Pediococcus*, or *Lactobacillus* genera) are involved in wine spoilage; significant amounts of lactic acid and acetic acid can be produced by metabolism of sugars, glycerol, tartaric or citric acid in the wine.² Lactic acid and the detrimental by-products of lactic bacterial action can be largely prevented by filtration and increased sulfite concentration.³

Clarification and Decolorization: Consider decolorizing if unusually high Sample Blank absorbance is obtained. Mix 10mL juice and approximately 0.1g polyamide powder or polyvinyl-polypyrrolidone (PVPP), stir for 1 minute and filter.

Lactic Acid Determination in Fermentation Samples: Due to enzyme content of some samples, absorbance may be affected by secondary (i.e. "creep") reactions in some samples. To inactivate endogenous enzymes, centrifuge the sample if necessary, alkalize to pH 8-10 with 1N NaOH, and place in 80°C water bath for 15 minutes. Centrifuge and use the supernatant in the assay.

Esterified Lactic Acid Determination: Both free Lactic acid and its esters may be measured in wine following alkaline hydrolysis. Heat 20mL of wine and 2mL sodium

hydroxide (2M) for 15 minutes while stirring under a reflux condenser. After cooling, neutralize with 1M sulfuric acid; quantitatively transfer to volumetric flask and Q.S. with distilled water to 50mL. Determine total Lactic acid and then calculate Lactic Acid esters (i.e. total Lactic Acid – free Lactic Acid.)

QUALITY CONTROL

A low and high level control should be included in each set of assays. Commercially available control material with established Lactic acid values may be used for quality control. Factors that may affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

Notes

1. Wavelength: The NADH absorbance maximum is 340nm; 334-340nm analysis provides a measuring range 0.03 – 0.35 g/L, while 365nm analysis provides a broader 0.06 – 0.7 @ 365nm but less sensitive measuring range.
2. Select standards within the assay range.

REFERENCES

1. Gutmann, I. & Wahlefeld, A.W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U.,ed.) 2nd ed., vol. 3, pp 1464-1468, Verlag Chemie, Weinheim, Academic Press, Inc., New York.
2. *The Home Winemakers Manual*
3. Peynaud, E. p.41, *Eng.Trans*, John Wiley & Sons, 1984.

Instructions LLA-Flex 15 Mar 2003

Manufactured by: **Unitech Scientific LLC**

12026 Centralia Road Suite H, Hawaiian Gardens, CA 90716

Tel: 562-924-5150 Fax: 562-809-3140

www.unitechscientific.com

email: info@unitechscientific.com