

D/L-LACTIC ACID

Enzymatic UV-Method

Product #: DLLA-F150 (75 Tests)

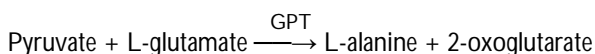
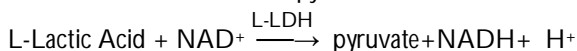
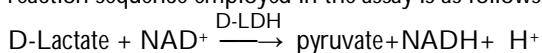
DLLA-F500 (250 Tests)

INTENDED USE

Unitech Scientific D/L-Lactic Acid FLEX-Reagents™ are intended for the sequential or simultaneous determination of D- and 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. D- and L-lactate dehydrogenase (D-LDH and L-LDH) catalyze the oxidation of D-lactate and L-lactate, respectively, 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

D/L-Lactic Acid FLEX-Reagent active ingredients are:

	Concentration	Quantity/Kit	
	as Formulated	75T	250T
1. <u>Mali-Lactic Buffer</u>			
Glycylglycine	1.5 M	50mL	170mL
L-Glutamic Acid	100 mM		
Stabilizers, pH 10			
2. <u>NAD a. Powder</u>	55 mM final		
b. NAD Diluent		14mL	47mL
3. <u>GPT Solution</u>	0.9 KU/mL	3.3mL	11mL
4. <u>D-LDH Solution</u>	3.2 KU/mL	3.3mL	11mL
5. <u>L-LDH Suspension</u>	2.7 KU/mL	3.3mL	11mL
6. <u>D-Lactic Acid Standard</u>	0.2G/L	2mL	2mL
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).		
* D-LDH Suspension (#4)	40 uL (1 drop)	40 uL (1 drop)
**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.

* Determine the lactate isomeric form in lower concentration first; i.e. add L-LDH suspension first for samples where L-lactic acid is lower than D-lactic acid concentration.

** If only total D/L-lactic acid determination is required, eliminate the incubation and A₂ absorbance readings.

- Dispense WRgt, mix and incubate 5 minutes. Zero spectrophotometer with the Reagent Blank cuvette. Read the ABS₁ values.
- Gently mix the D-LDH Suspension and dispense as shown above. Mix each cuvette, incubate and read the ABS₂ values.
- Gently mix the L-LDH Suspension and dispense as shown above. Mix each cuvette, incubate and read the ABS₃ (final absorbance) values.

CALCULATIONS

1. Calculate the absorbance difference for the sample:

$$A_2 - A_1 = \Delta \text{ABS}_{\text{D-lactate}}; A_3 - A_2 = \Delta \text{ABS}_{\text{L-lactate}}$$

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.

2. Compute Lactic Acid Levels by one of the following:

a. A single point standard, for example 0.2 G/L.

$$\begin{aligned} \text{Lactic Acid, G/L} &= \text{Conc. Std.} \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}} \\ &= 0.2 \times \frac{\text{Net } A_{\text{SAMPLE}}}{\text{Net } A_{\text{STANDARD}}} \end{aligned}$$

b. A multi-point standard curve run with each assay. Sample concentrations are calculated from the best-fit standard curve.

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 CO_2 (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-polyrrrolidone (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 DLLA-Flex 15Mar 2003

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