MA-0123

L-Lactate Assay II

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Background Information:

L-Lactate plays important roles in many biological processes. High concentrations are associated with disease states such as diabetes and lactic acidosis. Fundamentally this can be due to impaired tissue oxygenation, either due to a defect in mitochondrial oxidation processes or due to decreased delivery of oxygen to the various tissues. L-Lactate is the major lactate stereoisomer formed in human metabolism. D-Lactate is also present at much lower levels (~1-5% of L-Lactate). In this Lactate Assay, lactate is utilized by lactate dehydrogenase to generate NADH which is used to reduce a nearly colorless tetrazolium probe to produce an intensely colored formazan (λ_{max} = 450 nm). This assay is useful for detecting L(+)-Lactate at concentrations of 0.05 to 20 mM in a wide range of samples including serum or plasma, cells, culture and fermentation media without any pretreatment or purification.

Assay Principle:

1 – L-lactate is oxidized by L-Lactate Dehydrogenase, forming pyruvate and NADH
2 – NADH transfers an electron to a tetrazolium converting it to a formazan

Assay Components:

Assay Buffer	25 ml	WM	MA-0123A
Lactate Dehydrogenase/GPT Mix	lyoph	Green	MA-0123B
WST-8 Reagent	lyoph	Red	MA-0123C
L(+)-Lactate Standard	100 µl	Yellow	MA-0123D

Storage and Handling: Store kit at -20°C. Briefly centrifuge small vials prior to opening.
Assay Buffer: Warm to room temperature before use. Store at 4°C.
Lactate Dehydrogenase/GPT Mix: Reconstitute with 220 µl of Lactate Assay Buffer and mix thoroughly.
NAD/Tetrazolium Mix: Reconstitute with 220 µl of DI water and mix thoroughly.
Lactate Standard: 40 mM**,** ready to use as supplied.

Lactate Assay Protocol:

1. **Standard Curve Preparations:** Add 10 µl of the Lactate Standard to 990 µl Assay Buffer, giving a 400 µM solution, mix well. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl to a series of wells of a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer giving 0 – 2 – 4 – 6 – 8 – 10 nmol per well of L-Lactate Standard.

2. **Sample Preparation:** Homogenize tissue (10 mg) or cells (10^6) directly in 100 µl of assay buffer. Centrifuge at 16,000 X g to remove particulates. Transfer the clear supernatant to a fresh tube. For serum, use 0.5-10 µl. Unknown readings must be within the range set by the standard curve. If any samples are outside that range, dilute and rerun.

Notes:
- NADH or NADPH from cell or tissue extracts creates background in the assay. To correct for background, test the same amount of sample in the absence of Lactate Enzyme Mix. Subtract background readings from sample readings.
- Endogenous Lactate Dehydrogenase (LDH) may degrade lactate. Store samples containing LDH (culture medium, tissue lysate) at -80°C.
- Samples can be filtered through a 10 kDa MW centrifugal filter to remove protein.

3. **Reaction Mix:** Each reaction requires 50 µl. Prepare sufficient Reaction Mix for the number of sample and standard wells to be measured:

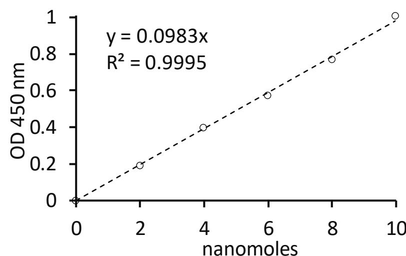
	Reaction Mix
Lactate Assay Buffer:	46 µl
NAD/Tetrazolium Mix:	2 µl
LDH/GPT Mix:	2 µl

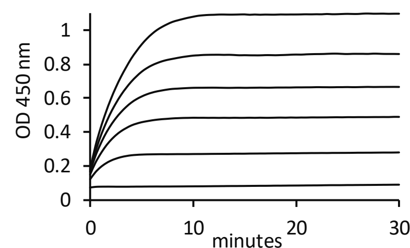
Mix and add 50 µl to each well.

4. Measurement: Monitor the reaction progress by absorbance (OD 450 nm). When the signal from the standards is no longer changing, the reaction is finished. It should take less than 60 minutes to reach the endpoint. The assay is faster if run at 37°C (optional)

5. Typical Results:

nmol Standard	Standard Raw Values	Background Corrected Values
	OD	
0	0.0911	0
2	0.2807	0.1896
4	0.4898	0.3987
6	0.6660	0.5749
8	0.8596	0.7685
10	1.0965	1.0054





6. Calculation: Subtract 0 Standard reading from standard readings. Plot the Lactate Standard Curve. Determine the slope of the standard curve. The slope defines the OD/nmol Lactate. Subtract any background control wells from paired test wells to get corrected signal due to lactate. Apply the slope of the standard curve to each corrected test value to determine nmoles of lactate in the well. To determine the amount of lactate in the original sample:
Total Lactate from sample = nmoles Lactate in the well X (total volume of deproteinized sample/volume added to the well)
Concentration of Lactate in original sample = Total Lactate from sample / mg (ml) of (liquid) sample.

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