MA-0115

L-Lactate Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

L-lactate is the primary end product of anaerobic metabolism. It accumulates when its consumption via the respiratory chain is slower than glycolysis, driven by the immediate need for energy. The Lactate pool can either be oxidized to pyruvate and further processed via mitochondrial oxidative phosphorylation generating ATP or it can be converted back to glucose via gluconeogenesis. Lactate levels range from 1-2 mM at rest up to 25 mM during intense exercise. Besides being a metabolic fuel, lactate has been discovered to be a feedback regulator and a signaling molecule in its own right. Abnormally high lactate is caused either by enhanced production or impaired utilization due to one of several diseases. The AkrivisBio Lactate Assay is a simple, sensitive method of measuring L-lactate in a variety of biological samples. In the assay, lactate is oxidized with the formation of hydrogen peroxide, which is further utilized by peroxidase to oxidize ADHP tp resorufin, resulting in intense color and fluorescence.

Assay Principle:

1 – L-lactate is oxidized by L-lactate Oxidase, forming pyruvate and hydrogen peroxide
2 – Hydrogen peroxide is used by peroxidase to oxidize ADHP, forming resorufin with intense color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0115A
ADHP Solution	200 µl	Red	MA-0115B
Lactate Oxidase/HRP	lyoph	Green	MA-0115C
L-Lactate Standard (40 mM)	100 µl	Yellow	MA-0115D

Storage and Handling: Store unopened kit at -20°C. Centrifuge all small vials for a few seconds before opening. Bring all components to room temperature before use.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: Ready to use as supplied. DMSO freezes just below room temperature. Store at –20°C.
Lactate Oxidase/HRP: Dissolve in 220 µl Assay Buffer. Store at -20°C.
L-Lactate Standard: Ready to use as supplied.

Assay Protocol:

1. Standard Curve Preparation: Add 10 µl of the Lactate Standard to 990 µl of Assay Buffer giving a 400 µM solution.
For an absorbance-based assay: 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.
For a fluorescence-based assay: Add 20 µl of the Lactate Standard to 180 µl Assay Buffer, mix, then take 10 µl of the diluted standard and add it to 990 µl of Assay buffer. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl of the 40 µM standard to a series of wells of a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0, 20, 40, 60, 80 & 100 pmol per well of the standard.

2. Sample Preparation: Deproteinize samples immediately (See PI-0102, PI-0103) after tissue/cell/biological fluid isolation to inactivate any enzymes present. Centrifuge briefly to remove any particulates or cell debris. Dilute deproteinized serum samples 10X. Add samples (5 – 50 µl) to wells in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer.

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

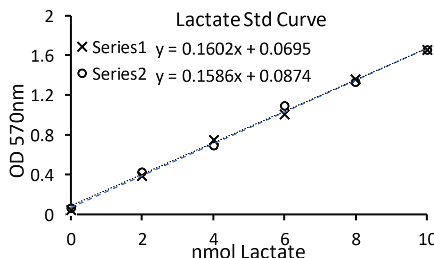
	Reaction Mix
Assay Buffer	46 µl
Lactate Oxidase/HRP	2 µl
ADHP Solution	2 µl

Mix and add 50 µl to each well

4. Measurement: Monitor the reaction progress with either absorbance (OD 570 nm) or fluorescence (Excitation/Emission = 535/587 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.

5. Typical Results:

nmol Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.0504	0.0626	0	0
2	0.3887	0.4297	0.3383	0.3671
4	0.7533	0.6962	0.7029	0.6336
6	1.0117	1.1012	0.9613	1.0386
8	1.3598	1.3324	1.3094	1.2698
10	1.6587	1.6602	1.6083	1.5976



6. Calculation: Subtract 0 Standard reading from standard readings. Plot the Lactate Standard Curve. Determine the slope of the standard curve. This defines the OD/nmol Lactate. Apply the slope of the standard curve to each test sample 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 of sample (ml of liquid sample)

Notes:
For samples with a high background, run each sample in pairs, with one of the pair used as a background control. Omit the Lactate Oxidase/HRP from the Reaction Mix for those background control wells. Before applying the standard curve slope to the test sample, subtract the signal from the paired background control.
Occasionally, a matrix effect is observed due to interference of the reaction by compounds present in the sample. This can be addressed by running each test sample in pairs, adding a known amount of lactate e.g, 2 nmol to one of the pairs. The difference in OD between the pairs is the response from 2 nmol lactate, similar to the slope of the standard curve in the regular assay. This OD/2 nmol can be applied to the sample without added lactate to determine the amount present in the sample.

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