MA-0148

D-Lactate Assay

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

Introduction:

D-lactate, an enantiomer of the common L-lactate, is primarily produced by gut bacteria. When the balance of gut bacteria is disrupted, as seen in conditions such as short bowel syndrome, intestinal bacterial overgrowth, or certain surgical interventions, D-lactate levels can rise abnormally. This elevation can lead to D-lactic acidosis, a condition characterized by an accumulation of D-lactate in the blood, which can lead to neurological symptoms. D-lactate measurements can be used as an indicator of intestinal health and bacterial overgrowth, especially in patients with altered gut anatomy or function. AkrivisBio's D-Lactate Assay is a simple, sensitive way to measure D-Lactate levels in a variety of biological samples.

Assay Principle:

1 – D-Lactate is oxidized by D-Lactate Dehydrogenase, forming NADH
2 – The NADH reducing equivalents are transferred to WST-8, a nearly colorless tetrazolium which is converted to a highly colored formazan.

Assay Components:

Assay Buffer	25 ml	WM	MA-0148A
D-Lactate Dehydrogenase	lyoph	Green	MA-0148B
WST-8 Reagent	lyoph	Red	MA-0148C
D-Lactate Standard	100 µl	Yellow	MA-0148D

Storage and Handling Considerations:

Store unopened assay at -20°C**.** Centrifuge all vials briefly before opening.
Assay Buffer: Ready to use as supplied. Bring to room temperature before use. Store at 4°C
D-Lactate Dehydrogenase: Reconstitute with 220 µl of Assay Buffer. Aliquot to conveniently sized portions and store at -20°C
WST-8 Reagent: Reconstitute with 220 µl of Assay Buffer. Store at 4°C.
D-Lactate Standard: Ready to use as supplied. Comes as a 4 mM solution. Store at 4°C.

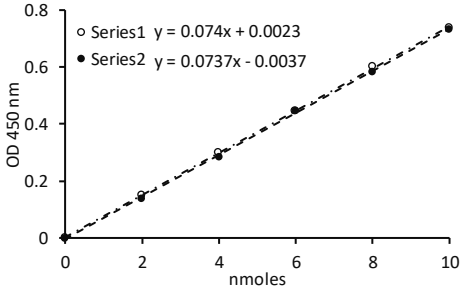
Assay Protocol:

1. Standard Curve: Dilute the 4 mM D-Lactate Standard to 0.4 mM by adding 10 µl of the Standard to 90 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0, 2, 4, 6, 8, 10 nmol of the D-Lactate Standard.
2. Samples: Liquid samples (serum, urine, saliva, etc. can be used directly. Homogenize tissue (10 mg) or cells (10^6), centrifuge at 16,000 X g for 10 minutes and transfer the clear supernatant to a fresh tube. Transfer up to 50 µl of each sample to wells of a 96-well plate and adjust all well volumes to 50 µl with Assay Buffer.
Note; NADH in extracted cells or tissues can cause a background. For these samples, prepare paired background control wells containing the same amount of sample.
3. Initiate Reaction: Each standard and sample well will require 50 µl of Reaction Mix. Prepare sufficient material for the total number of cells to be analyzed, containing:

	Reaction Mix	Background Control Mix
Assay Buffer	46 µl	48 µl
WST-8 Reagent	2 µl	2 µl
D-Lactate Dehydrogenase	2 µl	------

Add 50 µl of Reaction Mix to all standard and sample wells. Add 50 µl of Background Control Mix to any background control wells.
4. Measure: Place the plate in a plate reader and monitor the reaction progress at 450 nm for up to 30 min at room temperature. (The reaction should be completed within around 15 minutes.)
5. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0775	0.0868	0	0
2	0.2277	0.2261	0.1502	0.1393
4	0.3786	0.3727	0.3011	0.2859
6	0.5235	0.5338	0.4460	0.4470
8	0.6778	0.6718	0.6003	0.5850
10	0.8151	0.8196	0.7376	0.7328



6. Calculation: Subtract the zero Standard from all other standard values. Plot the Standard Curve and determine the Slope of the standard curve. This defines the sensitivity of the system. Subtract any Background Controls from their paired sample well values. If there are no Background Control wells, subtract the zero Standard value from sample well values. This corrects the absorbance for any non-D-Lactate derived color. Divide the background corrected sample well values by the slope of the standard curve to get nmoles in the well for all sample wells. To convert back to nmoles per original sample:
A - Divide the nmoles per well by the volume in µl of sample added to the well = nmoles D-Lactate per µl of sample.
B – Multiply the nmoles D-Lactate per µl of sample by the total volume in µl of sample of supernatant in step 2 above = total nmoles in sample
C – Divide total nmoles in sample by mg of tissue or # of cells processed = nmoles of D-Lactate per mg of tissue (or per # of cells, etc.)

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