Rev. 1

ScienCell™ Research Laboratories

L-Lactate Assay (LAC)

Catalog #8308

100 tests in 96-well plate

Product Description

Lactate is an important intermediary in glucose metabolism. Under hypoxic or anaerobic conditions, lactate dehydrogenase (LDH) converts pyruvate, the final product of glycolysis, to lactate. Monitoring lactate levels is an adequate indicator of the balance between tissue oxygen demand and utilization, and is useful when studying cellular physiology. This colorimetric assay is based on the reduction of the tetrazolium salt, INT, in a NADH-coupled enzyme reaction to formazan, which exhibits an absorbance maximum at 490 nm. The intensity of the optical density is proportional to the lactate concentration.

Kit Components

Cat. #	# of Vials	Reagent	Quantity	Storage
8308a	1	Assay Buffer	25 mL	4°C
8308b	1	L-Lactate Standard	1 mL	-20°C
8308c	1	Lyophilized Enzyme Mix	1 vial	-20°C, Dark
8308d	1	Substrate Mix	5 mL	-20°C
8308e	1	Reconstitution Solution	0.2 mL	4°C

Material Supplied by User

10 kDa MW spin filter (Millipore Sigma Cat. #UFC501008) or 0.5 M metaphosphoric acid (Sigma-Aldrich, Cat. #239275)

Quality Control

L-Lactate Assay is applied to serially diluted L-Lactate Standards with concentrations ranging from 0.0625 to 2 mM and is used to generate a standard curve (Figure 1).

Product Use

L-Lactate Assay (LAC) kits can be used to measure the lactate level of samples from cells, serum, plasma, cell culture media, and tissue extracts. LAC is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

All components are shipped on dry ice.

Storage

Upon receipt, store the Assay Buffer (Cat. #8308a) and Reconstitution Solution (Cat. #8308e) at 4°C.

Store L-Lactate Standard (Cat. #8308b), Enzyme Mix (Cat. #8308c), and Substrate Mix (Cat. #8308d) at -20°C.

Procedure (96-well plate)

A. Preparation of reagents

1. Reconstitute the lyophilized Enzyme Mix (Cat. #8308c) by adding 200 µL of the Reconstitution Solution (Cat. #8308e) to the Enzyme Mix (Cat. #8308c) vial. Mix well and store at -20°C in the dark until use.
2. Add 50 µL of L-Lactate Standard (Cat. #8308b) to 200 µL of Assay Buffer (Cat. #8308a) to make a 250 µL solution of 4 mM L-Lactate.
3. Obtain 7 test tubes, add 200 µL of Assay Buffer (Cat. #8308a) into each tube and label them #1 through #7.
4. Add 200 µL of the 4 mM L-Lactate solution into tube #1 and mix well to get the 2 mM L-Lactate standard.
5. Transfer 200 µL of the 2 mM L-Lactate standard from tube #1 to tube #2 and mix well to get the 1 mM L-Lactate standard.
6. Repeat step 5 for tubes #3-6 to serially dilute the L-Lactate standards. Do not add any L-Lactate to tube #7, which serves as blank.
7. Obtain a 96-well test plate and prepare 3 replicates (A, B, and C) of each L-Lactate standard by aliquoting 50 µL/well of each L-Lactate standard into triplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	2 mM	1 mM	0.5 mM	0.25 mM	0.125 mM	0.0625 mM	Blank
B	2 mM	1 mM	0.5 mM	0.25 mM	0.125 mM	0.0625 mM	Blank
C	2 mM	1 mM	0.5 mM	0.25 mM	0.125 mM	0.0625 mM	Blank

Note: Saturation is possible at concentrations over 0.5 mM. Additional standard concentrations can be added if necessary.

B. Preparation of test samples

1. Cells or tissue can be homogenized in 4 volumes of the Assay Buffer (Cat. #8308a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Endogenous NADH or NADPH from cell or tissue extracts would generate background for the lactate assay. To remove the NADH or NADPH background, the same amount of sample can be tested in the absence of the Enzyme Mix (Cat. #8308c).
3. Endogenous LDH may degrade lactate. Samples containing LDH (such as cell or tissue lysate) should be deproteinized with a 10 kDa MW spin filter (Millipore Sigma Cat. #UFC501008) or 0.5 M metaphosphoric acid (Sigma-Aldrich, Cat. #239275) to remove LDH and stored at -80°C until ready to use.
4. Samples should be serially diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 50 µL/well on the 96-well flat bottom plate.

C. Measurements

1. Add 2 µL of reconstituted Enzyme Mix (Cat. #8308c) into each well of the 96-well test plate containing L-Lactate standard, test samples and blank, without adding reconstituted Enzyme Mix (8308c) into the well containing test samples for control.
2. Add 50 µL of Substrate Mix (Cat. #8308d) into each well of the 96-well test plate containing the L-Lactate standard, test samples, blank, and test samples for control. Incubate for 20 minutes at room temperature in the dark.
3. Read the absorbance at 490 nm with an ELISA plate reader.

D. Calculations

1. Average the OD_{490nm} of replicate wells of each L-Lactate standard, test sample and blank. Subtract the average OD_{490nm} value of the blank from the average OD_{490nm} values obtained with all other standard and samples to get each absolute OD_{490nm} value.
2. Make a standard curve (shown in Figure 1) using the absolute OD_{490nm} values of the L-Lactate standard by plotting absolute OD_{490nm} as a function of L-Lactate concentration. Determine the equation and R^2 value of the trend line.
3. For samples requiring a control, subtract the absolute OD_{490nm} value without the enzyme from the absolute OD_{490nm} value with the enzyme and use this calibrated OD_{490nm} value to determine the sample L-Lactate concentration using the standard curve.
4. Calculate the L-Lactate concentration of test samples by using the equation for the trend line of the standard curve (y = Ax + B):

$$\text{L-Lactate concentration} = \frac{\text{calibrated } OD_{490nm}}{A}$$

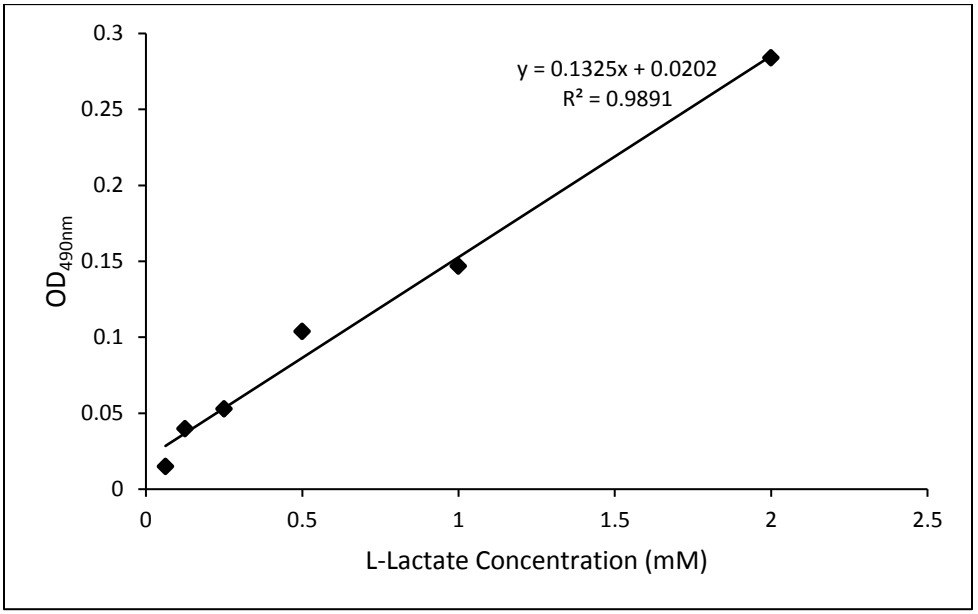

Figure 1. An example standard curve generated using the L-Lactate Assay.