L-Lactate Assay (LAC)

Cat. No. 8308
100 Tests in 96-well plate

Introduction

L-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, therefore, a good indicator of the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology. This colorimetric assay is based on the reduction of the terazolium salt INT in a NADH-coupled enzyme reaction to formazan, which exhibits an absorbance maximum at 490 nm. The intensity of the absorbance is proportional to the lactate concentration.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8308a	1	Assay buffer	25mL	4°C
8308b	1	L-Lactate standard	1mL	-20°C
8308c	1	Enzyme mix	0.2mL	-20°C
8308d	1	Substrate mix	5mL	-20°C

Product Use

L-Lactate Assay kit could measure lactate level of samples from cells, serum, plasma, cell culture media and tissue extracts. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from L-Lactate Assay of lactate solutions with concentrations ranging from 0.05 to 2 mM show a linear relationship between OD_{490nm} and lactate concentration (Figure 1).

Shipping and Stability

The kit would be shipped on dry ice and the kit is stable for half a year when handled properly.

Procedure (96-well plate)

A. Preparation of L-Lactate standard

1. Add 50 µL of L-Lactate standard (8308b) to 200 µL of assay buffer (8308a) to make a 250 µL solution of 4 mM L-Lactate.
2. Obtain 7 test tubes, add 200 µL of assay buffer (8308a) into each tube and label them #1 through #7.
3. Add 200 µL of the 4 mM L-Lactate solution into tube #1 and mix well to get the 2 mM L-Lactate standard.
4. 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.
5. Repeat step 4 for tubes #3-6 to serially dilute the L-Lactate standards. Do not add any L-Lactate to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 3 replicates (A, B, 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

B. Preparation of test samples

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (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, same amount of sample can be tested in the absence of enzyme mix (8308c).
3. Samples should be serial 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.

Recommendation:

Endogenous LDH may degrade lactate. Samples containing LDH (such as cell or tissue lysate) should be deproteinized with a 10k Da MW spin filter (Millipore UFC501008) or 0.5M metaphosphoric acid (Sigma 239275) to remove LDH and kept at -80°C for storage.

C. Measurements

1. Add 2 µL of enzyme mix (8308c) into each well of the 96-well test plate containing L-Lactate standard, test samples and blank. Without adding enzyme mix (8308c) into the well containing test samples for control.
2. Add 50 µL of substrate mix (8308d) into each well of the 96-well test plate containing L-Lactate standard, test samples, blank and test samples for control, Incubate for 20 minutes at room temperature in 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 ΔOD_{490nm} value.
2. Based on the calibrated ΔOD_{490nm} of the L-Lactate standard, make a standard curve by plotting ΔOD_{490nm} as a function of L-Lactate concentration. (See Figure 1 for a typical standard curve.) Determine the equation and R^2 value of the trend line.
3. For samples requiring control without enzyme mix, subtract the ΔOD_{490nm} without enzyme value from the ΔOD_{490nm} with enzyme value and use this $\Delta\Delta OD_{490nm}$ value to determine the sample L-lactate concentration from the standard curve.
4. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the L-Lactate concentration of test samples as follows:

$$[\text{L-Lactate}] = \frac{\Delta\Delta OD_{490nm}}{A}$$

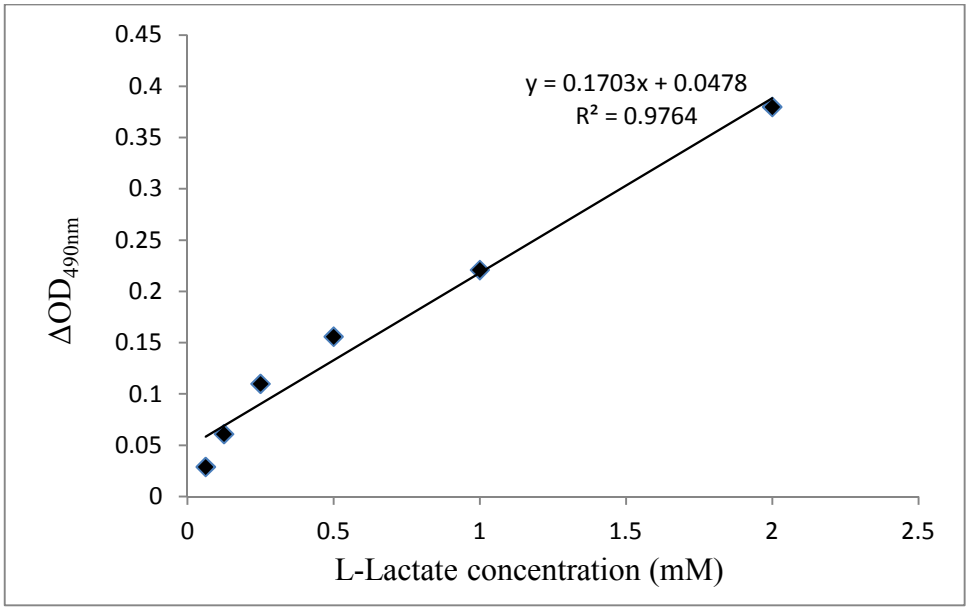

Figure 1. A typical L-Lactate standard curve measured by ScienCell™ L-Lactate Assay