Rev. 0

L-Lactate Assay- Fluorescence (LAC-FL)

Catalog #8788

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. The L-lactate assay relies on the LDH-catalyzed oxidation of lactate and the conversion of NAD to NADH. In a coupled reaction, the generated NADH reduces a probe, producing a highly fluorescent product. The fluorescence intensity, measured at an excitation wavelength of 560 nm and an emission wavelength of 590 nm, is directly proportional to the lactate concentration in the sample. The assay demonstrates a linear response up to 50 µM L-lactate, with a detection limit as low as 1 µM.

Kit Components

Cat. #	# of Vials	Reagent	Quantity	Storage
8788a	1	Assay Buffer	25 mL	4°C
8788b	1	L-Lactate Standard	0.2 mL	-20°C
8788c	1	Lyophilized Enzyme Mix	1 vial	-20°C, Dark
8788d	1	Substrate Mix	5 mL	-20°C
8788e	1	Probe	0.1 mL	-20°C, Dark
8788f	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 1 to 50 µM and is used to generate a standard curve (Figure 1).

Product Use

L-Lactate Assay- Fluorescence (LAC-FL) kits can be used to measure the lactate level of samples from cells, serum, plasma, cell culture media, and tissue extracts. LAC-FL 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. #8788a) and Reconstitution Solution (Cat. #8788f) at 4°C. Store L-Lactate Standard (Cat. #8788b), Lyophilized Enzyme Mix (Cat. #8788c), Substrate Mix (Cat. #8788d) and Probe (Cat. #8788e) at -20°C.

Procedure (96-well plate)

A. Preparation of reagents

1. Reconstitute the lyophilized Enzyme Mix (Cat. #8788c) by adding 200 µL of the Reconstitution Solution (Cat. #8788f) to the Enzyme Mix (Cat. #8788c) vial. Mix well and store at -20°C in the dark until use.
2. Add 10 µL of L-Lactate Standard (Cat. #8788b) to 240 µL of Assay Buffer (Cat. #8788a) to make a 250 µL solution of 100 µM L-Lactate.
3. Obtain 7 test tubes, add 200 µL of Assay Buffer (Cat. #8788a) into each tube and label them #1 through #7.
4. Add 200 µL of the 100 µM L-Lactate solution into tube #1 and mix well to get the 50 µM L-Lactate standard.
5. Transfer 200 µL of the 50 µM L-Lactate standard from tube #1 to tube #2 and mix well to get the 25 µM 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	50 µM	25 µM	12.5 µM	6.25 µM	3.12 µM	1.56 µM	Blank
B	50 µM	25 µM	12.5 µM	6.25 µM	3.12 µM	1.56 µM	Blank
C	50 µM	25 µM	12.5 µM	6.25 µM	3.12 µM	1.56 µM	Blank

B. Preparation of test samples

Note: The following substances interfere with the assay and should be avoided during sample preparation: EDTA (above 0.5 mM), ascorbic acid, SDS (above 0.2%), sodium azide, NP-40 (above 1%), and TWEEN® 20 (above 1%).

1. Cells or tissue can be homogenized in 4 volumes of the Assay Buffer (Cat. #8788a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Endogenous LDH may degrade L-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.
3. 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. Working Reagent Preparation and Measurements

1. Prepare enough reagents for the number of assays to be performed. For each well prepare 50 µL of working reagent by mixing 47 µL of Substrate Mix (Cat. #8788d), 2 µL of Enzyme Mix (Cat. #8788c), 1 µL of Probe (Cat. #8788e).
2. Quickly add 50 µL of working reagent to each well.
   Note: *Add working reagent without Enzyme mix into the well containing test samples for control.*
3. Tap the plate to mix and incubate for 20-30 minutes at room temperature in the dark.
4. Measure the fluorescence intensity (RFU) at excitation 530-560 nm, emission 580-600 nm.
5. If the sample's fluorescence intensity surpasses that of the 50 µM L-lactate standard or is higher than the internal standard, dilute the sample with purified water and rerun the assay.

D. Calculations

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

$$\text{L-Lactate } (\mu\text{M}) = \frac{\text{calibrated } RFU}{\text{A}} \times \text{DF}$$

DF= Dilution factor

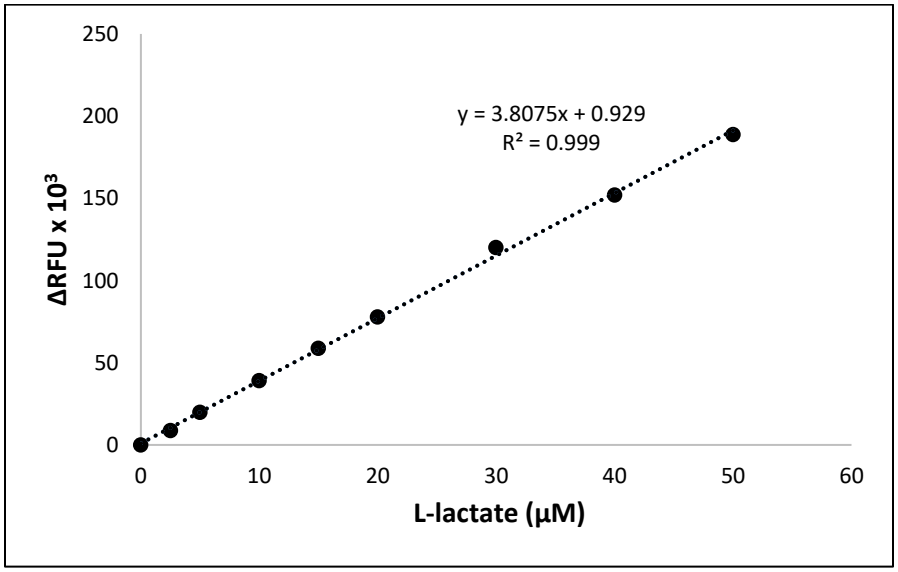

Figure 1. An example standard curve generated using the L-Lactate Assay-Fluorescence.