CREATIVE BIOMART®
Assay Kit

L-Lactate Colorimetric Assay Kit

Product Information

Cat

Kit-2412

Cat.No. Kit-2412

Description

Lactic acid, a major stereoisomer of lactate formed in human intermediary metabolism, has often been used as a physiological indicator for stress. Its determination in serum (normal range in blood 0.5-2.0 mM) is very important in the diagnosis and medical management of various diseases, such as tissue hypoxia, diabetes, circulatory failure and hematological disorders. Unlike a typical lactic acid-LDH assay, this new lactic acid assay kit provides a simple, reliable method for quantifying lactic acid in biological samples such as blood (serum and plasma), culture and fermentation media, etc. In the assay, lactic acid is oxidized by enzyme reactions to yield color product, which can be measured at 570 nm for colorimetric assay, And the color intensity is proportional to lactic acid concentrations, therefore the sample lactic acid concentration can be accurately calculated based on the lactic acid standards.

Applications

For biological research: L-Lactate measurement in biological samples
For drug/pharm research: Drug influence on L-Lactate metabolism

Storage

at -80°C

Shipping

Icepacks

Size

100 Assays

Kit Components

1. L-Lactate Assay Buffer(10x), 10ml

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2. L-Lactate Standard (10x), 100ul
3. Assay Probe, 45ul
4. L-Lactate Enzyme Mix, 55ul

Materials Required but Not Supplied

A plate reader capable of measuring absorbance at 570nm
Adjustable pipettes and a repeat pipettor
Distilled water(milliQ or HPLC-grade)
Clear Flat bottom 96-well plates if not included in the kit purchased

Detection method Colorimetric method at 570nm

Compatible Sample Types

Plasma,Serum,Cell culture supernatant, other body fluid

Preparation

Reagent Preparation
Note: All reagents are frozen. We recommend you spin small vials before opening.
1. L-Lactate Assay Buffer(10x)
Note: Please don't do dilution in the providedL-Lactate Assay Buffer bottle itself.
The bottle contains 10ml of 10x L-Lactate Assay Buffer. The assay buffer must be equilibrated to room temperature before use. Please make appropriate amount of 1xL-Lactate Assay Buffer for the assay, for example, by diluting 1ml of 10x L-Lactate Assay Buffer with 9 ml of distilled water. Prepare additional diluted assay buffer if needed. Store at -80°C.

2. L-Lactate Standard (10x)
Note: Please don't do dilution in the provided L-Lactate standard vial itself.
The vial contains 100µl of 1mM L-Lactate Standard. The standard must be equilibrated to room temperature before use. Dilute 100µl of 1mM L-Lactate Standard with 900µl of diluted L-Lactate Assay Buffer to prepare a 100µM L-Lactate Standard. 1ml of diluted standard is enough for making 3 standard curves if assayed in duplicate. Store at -80°C.

3. Assay Probe

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The vial contains 45µl of Assay Probe. It is sufficient for 100 wells. The assay probe must be equilibrated to room temperature before use.Protect from light. Store at -80°C.

4. L-Lactate Enzyme Mix
The vial contains 55µl of L-Lactate Enzyme Mix. The enzyme mix must be thawed on ice before use. It is sufficient for 100 wells. Store at -80°C.

Assay Protocol

Protocol
1.Sample Preparation
Blood, Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample (1/2; 1/4; 1/8; …) to ensure the readings are within the standard curve range. Your samples can be diluted with dH2O or diluted L-Lactate Assay Buffer.However, keep in mind if samples (such as hemolyzed serum/plasma) contain high level of lactate dehydrogenase capable of converting lactate to pyruvate, it is important for the samples to be deproteinated.

Add 50µl of samples to each well. We suggest that samples be assayed in duplicate.

Note:
If prepared samples are not assayed the same day, store the samples at -80°C. If samples need to be deproteinated, make sure to deproteinize the samples prior to storing in the freezer. The deproteinated samples will be stable for one month while stored at -80°C. For frozen samples, dilutions of samples must be done right before assaying.

2.Standard Curve Prepation
Add 50µl, 40µl, 30µl, 20µl, 10µl, 5µl, 1 µl, and 0µl of diluted L-Lactate Standard to each well, then adjust volume to 50µl/well with diluted L-Lactate Assay Buffer.
We suggest that L-Lactate Standards be assayed in duplicate. A standard curve has to be included with samples in each assay.

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3.Reaction Solution Preparation
50µl Reaction Solution is required for each well, and 1ml of Reaction Solution is prepared with the followings:
982µlL-Lactate Assay Buffer(1x)
10µlL-Lactate Assay Enzyme Mix
8 µl-Assay Probe
1ml of Reaction Solution is enough for ~20 wells.
Prepare appropriate amount of Reaction Solution for the number of assays to be performed.

Note: Please do not keep any leftover Reaction Solution as it has to be made fresh right before you run assays. If old Reaction Solution is used , assays will not work.

4.Perform the assay
a. Mix the reaction solution well and add 50µl of the reaction solution to each well containing the L-Lactate standards and test samples.
b. Incubate the reaction for 30 minutes in a 37°C incubator.
c. Measure absorbance at 570 nm in a microplate reader.

Assay time

30 minutes

Analysis

a. Average the OD570 nm values of replicate wells of each L-Lactate Standard, test samples, and blank. Then, correct background by subtracting the value derived from the blank from all sample readings.

b. Make a standard curve by plotting OD570 nm values from each L-Lactate Standards as a function of L-Lactate concentration. This can be done with excel spreadsheet.

c. Calculate the value of L-Lactate in samples using the equation obtained from the linear regression of the standard curve.

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L-Lactate(μM)= [(Corrected absorbance)-(y-intercept)]/Slope
Sensitivity
2μM-100μM

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