MANUAL

L-lactate Colorimetric Assay Kit (Lactate Oxidase Method)

Catalog #: MA-LAC

ISO 13485:2016

Introduction

Lactate is an intermediary product of carbohydrate metabolism and exists in the human body as two optical isomers: L-lactate and D-lactate. L-lactate is produced from pyruvate during anaerobic glycolysis and is present in blood at concentrations 100 times greater than D-lactate. Its concentration in the blood is dependent on the liver's metabolic rate, and its production by the erythrocytes and muscle cells. L-lactate has often been used as a physiological indicator for stress. Its determination in serum is very important in the diagnosis and medical management of various diseases, such as tissue hypoxia, diabetes, circulatory failure, and hematological disorders.

L-lactate Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool of measuring L-lactate concentration in plasma, serum, cell lysates, urine and other biological liquid samples. This assay employs a coupled enzymatic reaction system that combines multiple steps. Initially, lactate oxidase catalyzes the oxidation of L-lactate to pyruvate and hydrogen peroxide (H_2O_2). Peroxidase then catalyzes the reaction of hydrogen peroxide with the Colorimetric Probe to form a pink colored product. The optical density measured at 510nm is directly proportional to the concentration of L-lactate present in the sample.

Storage

The entire kit should be stored at < -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Assay Dilution Buffer	10 ml	2–8 °C, 6 months
L-lactate Standard	1 vial (100 µl of 4.9mM)	2–8 °C, 1 week
Enzyme Mix	2 amber vials (Lyophilized)	Do not store and reuse
Enzyme Buffer	25 ml	2–8 °C, 6 months
Colorimetric Probe	2 amber vials (Lyophilized)	Do not store and reuse

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 510 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Tubes to prepare sample dilutions
4. Incubator at 37°C
5. 50 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, normal human plasma has L-lactate concentrations in the range of 750-2000 µM. It is recommended to dilute the sample at least 1:2 with Assay Dilution Buffer before assaying.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, normal human serum has L-lactate concentrations in the range of 750-2000 µM. It is recommended to dilute the sample at least 1:2 with Assay Dilution Buffer before assaying.

4. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. The supernatant can be assayed directly.

Reagent Preparation

REAGENT	PREPARATION
Microplate (Item A)	No preparation
Assay Dilution Buffer	
Enzyme Buffer	
L-lactate Standard	See “Standard Preparation” section
Enzyme Mix	For each vial, reconstitute with 1 ml Enzyme Buffer.
Colorimetric Probe	For each vial, reconstitute with 1 ml Enzyme Buffer.
Working Solution	Add 1 mL reconstituted Enzyme Mix and 1 mL reconstituted Colorimetric Probe into 9 mL Enzyme Buffer to make the **Working Solution**. This is enough to assay 55 wells. Prepare the additional vial as needed. The Working Solution is stable for 1 day at 2-8°C in the dark.

Standard Preparation

To prepare a dilution series of L-lactate Standard in the concentration range of 0 µM – 2000 µM (see Table below),

1. Label 8 microtubes #1 through 8 which with the following concentrations: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 µM.
2. Pipette 59.5 µL Assay Dilution Buffer into labeled tube #1, and 40 µL Assay Dilution Buffer into labeled tube #2 – tube #8.
3. Pipette 40.5 µL of L-lactate Standard into tube#1, mix thoroughly and quick spin, to make a 2000 µM L-lactate Standard stock.
4. To make the 1000 µM standard, pipette 40 µL of tube #1 into the tube labeled #2. Mix thoroughly and quick spin.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 40 µl of the prior concentration until the 31.25 µM is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh and used immediately.

Labeled Tubes	L-lactate Standard (µL)	Assay Dilution Buffer (µL)	Standard Conc. (µM)	Standard Conc. (mg/dL)
1	40.5 µL of L-lactate Standard	59.5 µL	2000	40.49
2	40 µL of Tube #1	40 µL	1000	20.25
3	40 µL of Tube #2	40 µL	500	10.12
4	40 µL of Tube #3	40 µL	250	5.06
5	40 µL of Tube #4	40 µL	125	2.53
6	40 µL of Tube #5	40 µL	62.5	1.27
7	40 µL of Tube #6	40 µL	31.25	0.63
8	0µL	40 µL	0	0

Assay Procedure

Each L-lactate standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 5 µL of the diluted L-lactate Standards or samples to the 96-well microtiter plate.
2. Initiate the reaction by adding 200 µL Working Solution (See "Reagent Preparation") to each well.
3. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
4. Incubate the plate for 5 minutes at 37°C.
5. Measure the absorbance at 510nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 µM) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 510nm as a function of L-lactate concentration.

Determination of sample L-lactate concentration

L-lactate (µM) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

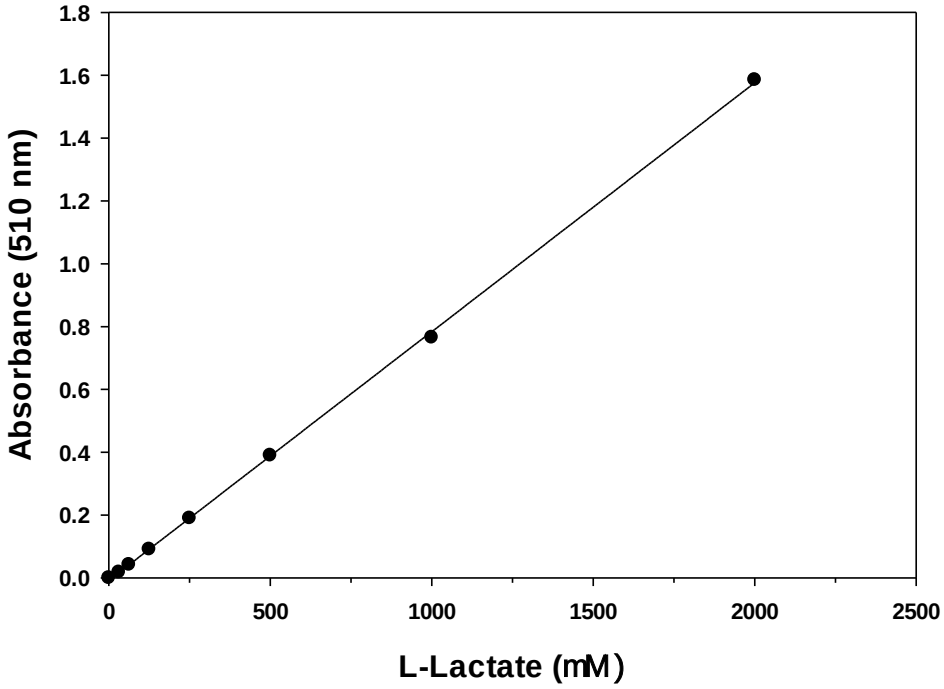
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 µM)

Slope is from the plot of L-lactate concentration vs. Absorbance shown in Typical data below

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated L-lactate concentration of the sample is higher than 2000 µM, dilute the sample in assay diluent solution and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.4%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 1.8%.

This product is for research use only.