



## L-Lactate Assay Kit

Item No. 700510

[www.caymanchem.com](http://www.caymanchem.com)

Customer Service 800.364.9897

Technical Support 888.526.5351

1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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## GENERAL INFORMATION

### Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, store the kit as supplied or remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700511	Lactate Assay Buffer (10X)	1 vial/10 ml	-20°C
700512	Lactate Cofactor Mixture	2 vials	-20°C
700513	Lactate Fluorescent Substrate	2 vials	-20°C
700514	L-Lactate Enzyme Mixture	2 vials	-20°C
700515	L-Lactate Standard	2 vials	-20°C
700518	MPA Assay Reagent	1 vial/2 g	RT
700517	Potassium Carbonate Assay Reagent	1 vial/5 ml	-20°C
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

Please read these instructions carefully before beginning this assay.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.) as some of them may be harmful. MPA (metaphosphoric acid) and potassium carbonate are corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

## If You Have Problems

### Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888  
Fax: 734-971-3641  
Email: techserv@caymanchem.com  
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed, on page 3, and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm
2. Adjustable pipettes and a multichannel or repeating pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

### Background

L(+)-Lactate is the major stereoisomer of lactate formed in human intermediary metabolism. The lactate to pyruvate ratio reflects the redox state of the cell and describes the balance between  $\text{NAD}^+$  and  $\text{NADH}$ , which is dependent on the interconversion of lactate and pyruvate *via* lactate dehydrogenase (LDH). LDH converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver.<sup>1,2</sup> Lactate is removed from cells *via* monocarboxylate transporters and is circulated to other tissues, such as muscle cells (skeletal and cardiac), the liver, and kidney, where it is used as a metabolic substrate.<sup>1</sup> At high concentrations of lactate, LDH exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased.

Lactic acidosis is the excess buildup of lactic acid in the body.<sup>3</sup> Lactic acidosis occurs when cells make lactic acid from glucose faster than it can be metabolized by the liver and kidneys.<sup>4</sup> The most common cause occurs during intense exercise or during convulsions where blood lactate levels can reach as high as 30 mM.<sup>5</sup> Lactic acidosis can also be caused by certain disease states such as sepsis, respiratory failure, HIV, cancer, diabetes, and kidney failure.<sup>1,6</sup> Prolonged exposure to high blood lactate levels (6-10 mM) results in high mortality rates. 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.

### About This Assay

Cayman's L-Lactate Assay provides a fluorescence-based method for detecting L-lactate in biological samples such as serum, plasma, blood, urine, and saliva. It can also be utilized to determine intracellular and extracellular lactate concentrations in cell culture samples. In the assay, lactate dehydrogenase catalyzes the oxidation of lactate to pyruvate, along with the concomitant reduction of  $\text{NAD}^+$  to  $\text{NADH}$ .  $\text{NADH}$  reacts with the fluorescent substrate to yield a highly fluorescent product. The fluorescent product is analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

## PRE-ASSAY PREPARATION

### Reagent Preparation

#### 1. Lactate Assay Buffer (10X) - (Item No. 700511)

This vial contains 10 ml of a 500 mM potassium phosphate, pH 7.5 solution. Mix 5 ml of Assay Buffer concentrate with 45 ml of HPLC-grade water. This final Assay Buffer (50 mM potassium phosphate, pH 7.5) is used in the assay. The diluted buffer is stable for six months at 4°C.

#### 2. Lactate Cofactor Mixture - (Item No. 700512)

This vial contains a lyophilized powder of lactate dehydrogenase cofactors. Reconstitute the contents of the vial with 1.2 ml of diluted Assay Buffer. This is enough cofactor mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted mixture is stable for two weeks at -20°C.

#### 3. Lactate Fluorescent Substrate - (Item No. 700513)

This vial contains a lyophilized powder of fluorescent substrate. Reconstitute the contents of the vial with 1.2 ml of diluted Assay Buffer. This is enough fluorescent substrate to assay 60 wells. Prepare the additional vial as needed. The reconstituted substrate is stable for two weeks at -20°C.

#### 4. L-Lactate Enzyme Mixture - (Item No. 700514)

This vial contains a lyophilized powder of enzymes. Reconstitute the contents of the vial with 2.5 ml of diluted Assay Buffer and put the vial on ice. This is enough enzyme mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted enzymes are stable for eight hours at 4°C or two weeks at -20°C.

#### 5. L-Lactate Standard - (Item No. 700515)

This vial contains a lyophilized powder of L-lactate. Reconstitute the contents of the vial with 1 ml of diluted Assay Buffer to prepare a 10 mM lactate stock. This stock is ready to use to prepare the standard curve. The reconstituted standard is stable for eight hours at room temperature.

#### 6. MPA Assay Reagent - (Item No. 700518)

This vial contains 2 g of metaphosphoric acid (MPA). Dissolve 1.6 g of MPA in 40 ml of HPLC-grade water to prepare a 0.5 M MPA stock for deproteinating the samples. Store the diluted acid solution at room temperature. The diluted acid is stable for three months at room temperature.

#### 7. Potassium Carbonate Assay Reagent - (Item No. 700517)

This vial contains 5 ml of a 5 M potassium carbonate solution. The reagent is ready to use as supplied.

## Sample Preparation

Due to the presence of lactate dehydrogenase in samples, care must be taken during sample processing to prevent the conversion of lactate to pyruvate. It is important for samples to be deproteinated upon collection, and then they can be stored at  $-80^{\circ}\text{C}$ .<sup>7</sup>

### Plasma

Typically, normal human plasma has a L-Lactate concentration in the range of 750-2,000  $\mu\text{M}$ .

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at  $25^{\circ}\text{C}$ . Pipette off the top yellow plasma layer without disturbing the white buffy layer.
3. In a 1.5 ml microcentrifuge tube, deproteinate 500  $\mu\text{l}$  of plasma by adding 500  $\mu\text{l}$  of cold 0.5 M MPA. Vortex the tube and place on ice for five minutes.
4. Centrifuge at 10,000 x g for five minutes at  $4^{\circ}\text{C}$  to pellet the proteins. Remove the supernatant and add 50  $\mu\text{l}$  of Potassium Carbonate to neutralize the acid.
5. Centrifuge at 10,000 x g for five minutes at  $4^{\circ}\text{C}$  to remove any precipitated salts. Remove the supernatant for assaying.
6. If not assaying the same day, freeze at  $-80^{\circ}\text{C}$ . The deproteinated plasma sample will be stable for one month while stored at  $-80^{\circ}\text{C}$ .
7. Dilute the sample 1:2-1:4 with Assay Buffer before assaying.

### Serum

Typically, normal human serum has a L-Lactate concentration in the range of 750-2,000  $\mu\text{M}$ .

1. Collect blood without using an anticoagulant.
2. Allow the blood to clot for 30 minutes at  $25^{\circ}\text{C}$ .
3. Centrifuge the blood at 2,000 x g for 15 minutes at  $25^{\circ}\text{C}$ . Pipette off the top yellow serum layer without disturbing the white buffy layer.
4. In a 1.5 ml microcentrifuge tube, deproteinate 500  $\mu\text{l}$  of serum by adding 500  $\mu\text{l}$  of cold 0.5 M MPA. Vortex the tube and place on ice for five minutes.
5. Centrifuge at 10,000 x g for five minutes at  $4^{\circ}\text{C}$  to pellet the proteins. Remove the supernatant and add 50  $\mu\text{l}$  of Potassium Carbonate to neutralize the acid.
6. Centrifuge at 10,000 x g for five minutes at  $4^{\circ}\text{C}$  to remove any precipitated salts. Remove the supernatant for assaying.
7. If not assaying the same day, freeze at  $-80^{\circ}\text{C}$ . The deproteinated serum sample will be stable for one month while stored at  $-80^{\circ}\text{C}$ .
8. Dilute the sample 1:2-1:4 with Assay Buffer before assaying.

## Blood

Typically, normal human blood has a L-Lactate concentration in the range of 750-2,000  $\mu\text{M}$ .

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. In a 1.5 ml microcentrifuge tube, deproteinate 500  $\mu\text{l}$  of blood by adding 500  $\mu\text{l}$  of cold 0.5 M MPA. Vortex the tube and place on ice for five minutes.
3. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50  $\mu\text{l}$  of Potassium Carbonate to neutralize the acid.
4. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
5. If not assaying the same day, freeze at -80°C. The deproteinated blood sample will be stable for one month while stored at -80°C.
6. Dilute the sample 1:2-1:4 with Assay Buffer before assaying.

## Saliva

1. Collect saliva in a clean test tube.
2. In a 1.5 ml microcentrifuge tube, deproteinate 500  $\mu\text{l}$  of saliva by adding 500  $\mu\text{l}$  of cold 0.5 M MPA. Vortex the tube and place on ice for five minutes.
3. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50  $\mu\text{l}$  of Potassium Carbonate to neutralize the acid.
4. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
5. If not assaying the same day, freeze at -80°C. The deproteinated saliva sample will be stable for one month while stored at -80°C.
6. The sample does not need to be diluted before assaying.

## Urine

1. Collect urine in a clean container.
2. In a 1.5 ml microcentrifuge tube, deproteinate 500 µl of urine by adding 500 µl of cold 0.5 M MPA. Vortex the tube and place on ice for five minutes.
3. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
4. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
5. If not assaying the same day, freeze at -80°C. The deproteinated urine sample will be stable for one month while stored at -80°C.
6. The sample does not need to be diluted before assaying.

*NOTE: L-Lactate values from urine samples can be standardized using Cayman's Creatinine (urinary) Assay Kit (Item No. 500701).*

## For Measurement of Extracellular and Intracellular L-Lactate

Collect cells (~ $10^6$ ) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). The supernatant will be used to quantify extracellular L-lactate (see below). The cell pellet is used for intracellular L-lactate determination (see page 16).

### Extracellular L-Lactate

1. In a 1.5 ml microcentrifuge tube, deproteinate 500 µl of supernatant by adding 500 µl of cold 0.5 M MPA. Vortex the tube and place on ice for five minutes.
2. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
3. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
4. If not assaying the same day, freeze at -80°C. The deproteinated sample will be stable for one month while stored at -80°C.
5. Dilute the sample 1:2 with Assay Buffer before assaying.



## Intracellular L-Lactate

1. Add 1 ml of PBS to the cell pellet to wash the cells. A cell count can be performed at this time.
2. Centrifuge at 10,000 x g for five minutes at 4°C. Discard the supernatant.
3. To prepare 0.25 M MPA, mix 1 ml of 0.5 M MPA with 1 ml of HPLC-grade water.
4. To deproteinate, add 0.5 ml of 0.25 M MPA to the cell pellet. Vortex the tube and place on ice for five minutes.
5. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 25 µl of Potassium Carbonate to neutralize the acid.
6. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
7. If not assaying the same day, freeze at -80°C. The deproteinated sample will be stable for one month while stored at -80°C.
8. Dilute the sample 1:2 with Assay Buffer before assaying.

## ASSAY PROTOCOL

### Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of L-lactate standards and samples to be measured in duplicate is given below in Figure 1. We suggest you record the contents of each well on the template sheet provided (see page 26).

	1	2	3	4	5	6	7	8	9	10	11	12
A	(A)	(A)	(S1)	(S1)	(S9)	(S9)	(S17)	(S17)	(S25)	(S25)	(S33)	(S33)
B	(B)	(B)	(S2)	(S2)	(S10)	(S10)	(S18)	(S18)	(S26)	(S26)	(S34)	(S34)
C	(C)	(C)	(S3)	(S3)	(S11)	(S11)	(S19)	(S19)	(S27)	(S27)	(S35)	(S35)
D	(D)	(D)	(S4)	(S4)	(S12)	(S12)	(S20)	(S20)	(S28)	(S28)	(S36)	(S36)
E	(E)	(E)	(S5)	(S5)	(S13)	(S13)	(S21)	(S21)	(S29)	(S29)	(S37)	(S37)
F	(F)	(F)	(S6)	(S6)	(S14)	(S14)	(S22)	(S22)	(S30)	(S30)	(S38)	(S38)
G	(G)	(G)	(S7)	(S7)	(S15)	(S15)	(S23)	(S23)	(S31)	(S31)	(S39)	(S39)
H	(H)	(H)	(S8)	(S8)	(S16)	(S16)	(S24)	(S24)	(S32)	(S32)	(S40)	(S40)

A-H = Standards

S1-S40 = Sample wells

Figure 1. Sample plate format

### Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

### General Information

- The final volume of the assay is 200  $\mu\text{l}$  in all the wells.
- All reagents except the Enzyme Mixture must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

### Standard Preparation

Label eight clean glass test tubes A-H. Add the amount of reconstituted L-Lactate Standard (10 mM) and diluted Assay Buffer to each tube as described in Table 1. The diluted standards are stable for four hours at room temperature.

Tube	L-Lactate Standard ( $\mu\text{l}$ )	Assay Buffer ( $\mu\text{l}$ )	Final L-Lactate Concentration ( $\mu\text{M}$ )
A	0	1,000	0
B	2.5	997.5	25
C	5	995	50
D	10	990	100
E	20	980	200
F	40	960	400
G	60	940	600
H	100	900	1,000

Table 1. Preparation of L-Lactate standards

## Performing the Assay

1. **Standard Wells** - add 20 µl of standard (tubes A-H) to the designated wells on the plate (see **Sample plate format**, Figure 1, page 17).
2. **Sample Wells** - add 20 µl of each sample to at least two wells.
3. Add 100 µl of diluted Assay Buffer, 20 µl of Cofactor Mixture, and 20 µl of Fluorometric Substrate to all wells being used.
4. Initiate the reactions by adding 40 µl of Enzyme Mixture to all of the wells being used.
5. Incubate the plate for 20 minutes at room temperature and then read the fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. The fluorescence is stable for up to 45 minutes.

## ANALYSIS

### Calculations

1. Determine the average fluorescence of each standard and sample.
2. Subtract the fluorescence value of the standard A (0 µM) from itself and all other standards and samples. This is the corrected fluorescence (CF).
3. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of the final concentration of lactate from Table 1. See Figure 2, on page 22, for a typical standard curve.
4. Calculate the L-lactate concentration of the samples using the equation below.

$$\text{L-Lactate } (\mu\text{M}) = \left[ \frac{\text{CF} - (\text{y-intercept})}{\text{Slope}} \right] \times 2^* \times \text{Sample dilution}$$

\*This is a dilution factor to correct for deproteinating the samples with 0.5 M MPA. Do not use this dilution factor when determining intracellular lactate values.

*NOTE: L-Lactate values from urine samples can be standardized using Cayman's Creatinine (urinary) Assay Kit (Item No. 500701).*

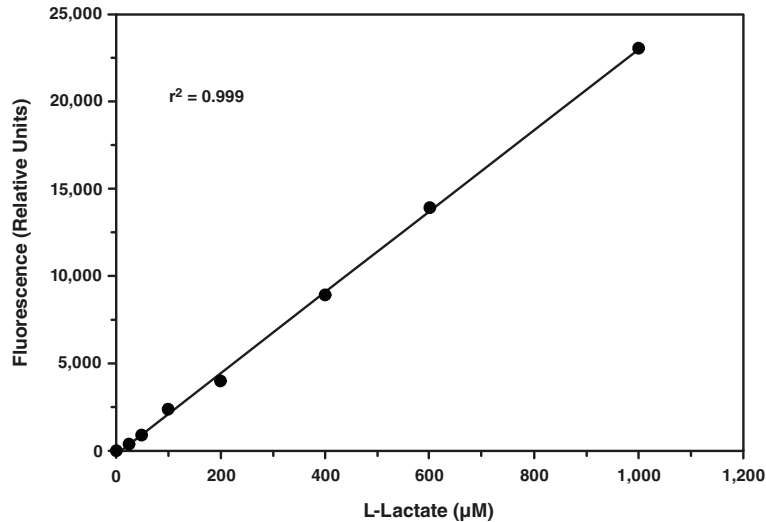


Figure 2. L-Lactate standard curve

## Performance Characteristics

### Precision:

When a series of eight deproteinated blood or eight deproteinated plasma measurements were performed on the same day, the intra-assay coefficient of variation was 2.4% and 3.5%, respectively. When a series of eight deproteinated blood or eight deproteinated plasma measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 3.5% and 3.8%, respectively.

### Assay Specificity:

To assess substrate specificity, the assay was performed with L-lactate replaced by structurally similar compounds. Compounds such as pyruvate, D-Lactate, and  $\alpha$ -keto acids might interfere with the measurement of L-Lactate. Therefore, fluorescence signals generated by L-Lactate and other  $\alpha$ -keto acids as substrates were evaluated under identical conditions. When testing the specificity of the assay, there was no interference by adding D-Lactate, pyruvate, phosphoenolpyruvate, 2-oxobutyrate, or oxaloacetate up to a final concentration of 1 mM.

## RESOURCES

### Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence detected above background in the sample wells	Sample was too dilute	Re-assay using a more concentrated sample
The fluorometer exhibited 'MAX' values for the wells	The <i>gain</i> setting is too high	Reduce the <i>gain</i> and re-read
The fluorescence of the sample wells were higher than the last standard	Sample was too concentrated	Dilute the sample and re-assay

### References

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	A	B	C	D	E	F	G	H

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman’s Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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