L-Lactate Assay Kit

Item No. 700510

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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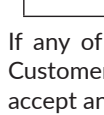
GENERAL INFORMATION

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 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) (Fluorometric Assay)	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

Email: techserv@caymanchem.com

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 or a plate reader with the ability to measure absorbance at 535 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
4. A clear bottom polystyrene plate if absorbance will be measured.

INTRODUCTION

Background

L-Lactate is the major stereoisomer of lactate formed from pyruvate by lactate dehydrogenase (LDH), using NADH as a cofactor, under hypoxic or anaerobic conditions.[1] L-Lactate can be converted back into pyruvate by LDH to enter gluconeogenesis under more oxygenated conditions. It circulates in the blood and is taken up by a variety of cells and tissues but is primarily used by skeletal and cardiac muscle as an alternate energy source.[1,2] Clearance occurs *via* the liver and kidneys.[2]

L-Lactate accumulates in a condition called lactic acidosis when the rate-limiting reactions in gluconeogenesis are impaired, when pyruvate utilization is disrupted, or under conditions that increase glycolysis, such as intense exercise, hypoxia, or disease states such as sepsis.[3] Lactic acidosis is associated with poor prognosis and increased mortality in patients with critical illnesses, sepsis, and liver failure, among others, and has been used as an indicator of worsening conditions in patients[2,3]

About This Assay

Cayman's L-Lactate Assay provides both a fluorescence and an absorbance-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, LDH catalyzes the oxidation of lactate to pyruvate, along with the concomitant reduction of NAD^+ to NADH. NADH reacts with the substrate to yield a final product that can be measured fluorometrically with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm, or colorimetrically at 535 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 solution. Mix 5 ml of Assay Buffer concentrate with 45 ml of pure water. This final Assay Buffer (1X) (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 LDH cofactors. Reconstitute the contents of the vial with 1.2 ml of Assay Buffer (1X). 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 Substrate - (Item No. 700513)**

   This vial contains a lyophilized powder of lactate substrate. Reconstitute the contents of the vial with 1.2 ml of Assay Buffer (1X). This is enough 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 Assay Buffer (1X) 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 2 ml of diluted Assay Buffer (1X) to prepare a 5 mM lactate stock solution. This stock solution 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 pure water to prepare a 0.5 M MPA stock solution 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 LDH 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°C.[4]

Plasma

Typically, normal human plasma has an L-lactate concentration in the range of 750-2,000 µM.

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°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer.
3. In a 1.5 ml microcentrifuge tube, deproteinate 500 µl of plasma by adding 500 µl 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°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
5. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
6. If not assaying the same day, freeze at -80°C. The deproteinated plasma sample will be stable for one month while stored at -80°C.
7. Dilute the sample 1:2-1:4 with Assay Buffer (1X) before assaying.

Serum

Typically, normal human serum has an L-lactate concentration in the range of 750-2,000 µM.

1. Collect blood without using an anticoagulant.
2. Allow the blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 25°C. Pipette off the top yellow serum layer without disturbing the white buffy layer.
4. In a 1.5 ml microcentrifuge tube, deproteinate 500 µl of serum by adding 500 µl 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°C to pellet the proteins. Remove the supernatant and add 50 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 serum sample will be stable for one month while stored at -80°C.
8. Dilute the sample 1:2-1:4 with Assay Buffer (1X) before assaying.

Blood

Typically, normal human blood has an L-lactate concentration in the range of 750-2,000 µM.

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. In a 1.5 ml microcentrifuge tube, deproteinate 500 µl of blood 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 blood sample will be stable for one month while stored at -80°C.
6. Dilute the sample 1:2-1:4 with Assay Buffer (1X) before assaying.

Saliva

1. Collect saliva in a clean test tube.
2. In a 1.5 ml microcentrifuge tube, deproteinate 500 µl of saliva 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 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.

It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

For Measurement of Extracellular and Intracellular L-Lactate

Collect cells (~10-24 x 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 (1X) 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 pure 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 (1X) 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 µl 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. Alternatively, the absorbance can be read at 535 nm.
- To monitor abosorbance, a clear bottom polysteyrene plate should be used (not supplied).

Standard Preparation

Label eight clean glass test tubes A-H. Add the amount of reconstituted L-Lactate Standard (5 mM) and Assay Buffer (1X) to each tube as described in Table 1. The diluted standards are stable for four hours at room temperature.

Tube	L-Lactate Standard (µl)	Assay Buffer (1X) (µl)	Final L-Lactate Concentration (µM)
A	0	1,000	0
B	5	995	25
C	10	990	50
D	20	980	100
E	40	960	200
F	80	920	400
G	120	880	600
H	200	800	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 Assay Buffer (1X), 20 µl of Cofactor Mixture, and 20 µl of the lactate 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. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate 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. Alternatively, the absorbance may be read at 535 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence or absorbance of each standard and sample.
2. Subtract the fluorescence or absorbance value of the standard A (0 µM) from itself and all other standards and samples. This is the corrected signal (CS).
3. Plot the CS values (from step 2 above) of each standard as a function of the final concentration of L-lactate from Table 1. See Figures 2 and 3, on pages 22 and 23, for typical standard curves.
4. Calculate the L-lactate concentration of the samples using the equation below.

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

*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.

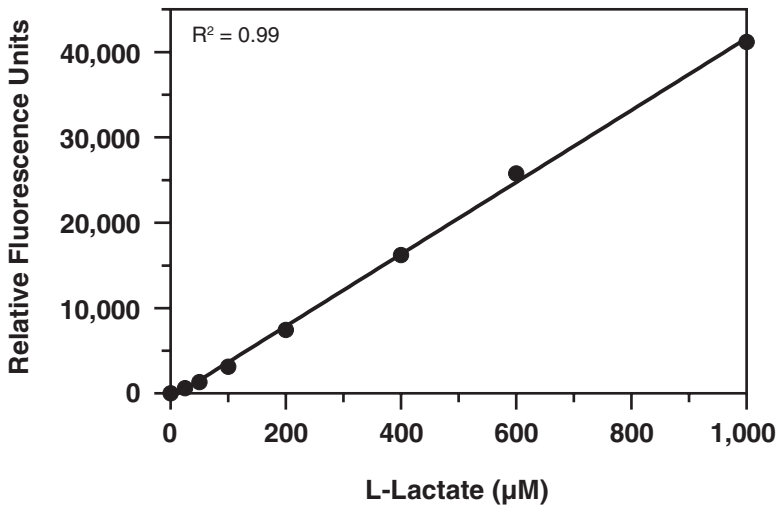


Figure 2. L-Lactate standard curve measured fluorometrically

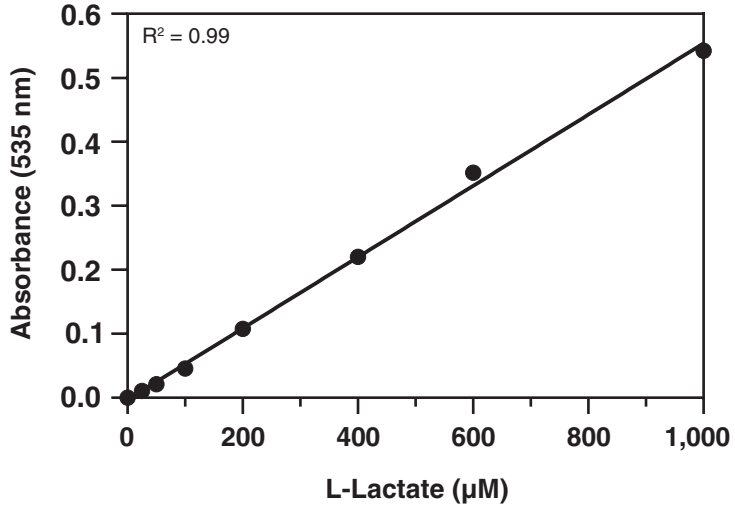


Figure 3. L-Lactate standard curve measured colorimetrically

Performance Characteristics

Precision:

When a series of eight deproteinated blood or eight deproteinated plasma mesurements were performed on the same day, the intra-assay coefficients of variation were 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 coefficients of variation were 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 α-keto acids might interfere with the measurement of L-lactate. Therefore, fluorescence signals generated by L-lactate and other α-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.

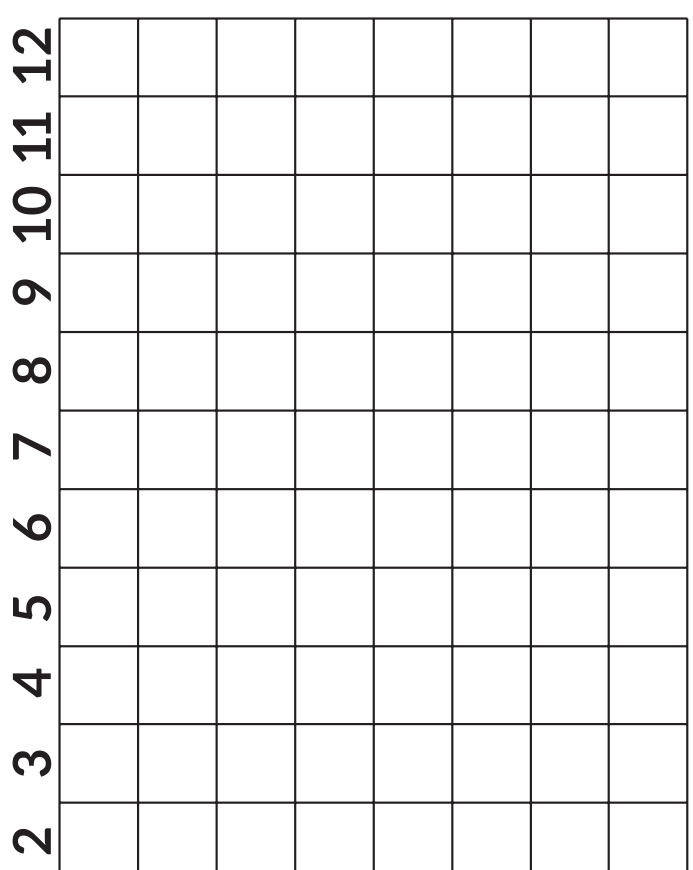
RESOURCES

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 signal 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 *gain* setting is too high	Reduce the *gain* and re-read
The signal of the sample wells were higher than the last standard	Sample was too concentrated	Dilute the sample and re-assay

References

1. Adeva, M., González-Lucán, M., Seco, M., *et al.* Enzymes involved in L-lactate metabolism in humans. *Mitochondrion* **13(6)**, 615-629 (2013).
2. Adeva-Andany, M., López-Ojén, M., Funcasta-Calderón, R., *et al.* Comprehensive review on lactate metabolism in human health. *Mitochondrion* **17**, 76-100 (2014).
3. Rattu, G., Khansili, N., Maurya, V.K., *et al.* Lactate detection sensors for food, clinical and biological applications: A review. *Environ. Chem. Lett.* **19**,1135-1152 (2021).
4. McLellan, A.C., Phillips, S.A., and Thornally, P.J. Flourimetric assay of D-lactate . *Anal. Biochem.* **206(1)**, 12-16 (1992).



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.