D-Lactate Assay Kit

Item No. 700520

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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
700521	D-Lactate Enzyme Mixture	2 vials	-20°C
700522	D-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

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

INTRODUCTION

Background

D(-)-Lactate is a stereoisomer of lactate and is present in blood at only 1-5% the concentration of L(+)-lactate. Exogenous sources of D-lactate include fermented foods such as yogurt, sauerkraut, and pickles. D-lactate is normally produced in the gastrointestinal tract, mainly by lactobacilli and bifidobacteria or is produced in the cytosol of cells by the glyoxylase pathway.[1] Methylglyoxal is converted to D-lactate and glutathione *via* the intermediate S-D-lactoylglutathione by glyoxalase I and glyoxalase II. L-Lactate is rapidly metabolized to pyruvate by L-lactate dehydrogenase in the liver. D-Lactate was thought to only be metabolized to pyruvate by D-α-hydroxy acid dehydrogenase, which metabolizes D-lactate at about one-fifth the rate that L-lactate dehydrogenase metabolizes L-lactate.[1] Mammals were originally reported to lack D-lactate dehydrogenase, an enzyme that had been isolated only in lower organisms. However, new studies have identified putative human and murine mitochondrial D-lactate dehydrogenases.[1,2]

D-Lactic acidosis has been defined as metabolic acidosis accompanied by an increase in serum D-lactate (≥3 mM) and is associated with neurotoxic effects.[1] D-Lactate production, accumulation, and acidosis are caused by excessive gastrointestinal fermentation of carbohydrate by lactobacilli, and the subsequent inability of the body to adequately clear D-lactate by the kidneys. D-Lactic acidosis is a rare metabolic occurrence in humans, but is occasionally observed as a consequence of short bowel syndrome or after jejunoileal bypass surgery.[1,3] It also occurs in ruminants after grain overfeeding. Elevated D-lactate levels have also been shown to occur in diabetes, infection, ischemia, and trauma.[1] D-Lactate has an important role in numerous aspects of monogastric metabolism, is clinically important in a variety of malabsorptive or gastrointestinal nutrient overload conditions, and may be important in some types of sepsis.[4] Monitoring D-lactate levels is useful when studying cellular and animal physiology.

About This Assay

Cayman's D-Lactate Assay provides a fluorescence-based method for detecting D-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, D-lactate dehydrogenase catalyzes the oxidation of D-lactate to pyruvate, along with the concomitant reduction of NAD^+ to NADH. NADH reacts with the fluorescent substrate to yield a highly fluorescent product. The fluorescent product can be 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)**

   The vial contains 10 ml of a 500 mM potassium phosphate, pH 7.5 solution. Dilute 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)**

   The 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)**

   The 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. **D-Lactate Enzyme Mixture - (Item No. 700521)**

   The vial contains a lyophilized powder of enzymes. Reconstitute the contents of the vial gently with 2.5 ml of diluted Assay Buffer and put the vial on ice. Do not vortex. 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.

5. **D-Lactate Standard - (Item No. 700522)**

   The vial contains a lyophilized powder of D-lactate. Reconstitute the contents of the vial with 1 ml of diluted Assay Buffer to prepare a 10 mM lactate stock. It is now 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)**

   The vial contains 2 g of metaphosphoric acid (MPA). To prepare 0.5 M MPA used for deproteinating the samples, dissolve 1.6 g of MPA in 40 ml of HPLC-grade water. 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)**

   The 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°C.[5]

Plasma

Typically, normal human plasma has a D-Lactate concentration in the range of 13-90 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. Transfer the top yellow plasma layer into a clean test tube 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. Collect 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. Collect 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.

Serum

Typically, normal human serum has a D-Lactate concentration in the range of 13-90 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. Transfer the top yellow serum layer into a clean test tube 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. Collect 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. Collect 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.

Blood

Typically, normal human blood has a D-Lactate concentration in the range of 13-90 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. Collect 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. Collect 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.

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. Collect 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. Collect 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. Collect 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. Collect 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 D-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 D-lactate (see below). The cell pellet is used for intracellular D-lactate determination (see below).

Extracellular D-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. Collect 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. Collect 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.

Intracellular D-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, dilute 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. Collect 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. Collect 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.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of D-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 22).

	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 repeating pipettor 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.

Standard Preparation

Take eight clean glass test tubes and mark them A-H. Add the amount of reconstituted D-lactate (10 mM) and Assay Buffer to each tube as described in Table 1, below. The diluted standards are stable for four hours at room temperature.

Tube	D-Lactate (µl)	Assay Buffer (µl)	Final Concentration (µM)
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 D-Lactate standards

Performing the Assay

1. **Standard Wells -** add 20 µl of standard (tubes A-H) per well in the designated wells on the plate (see **Sample Plate Format**, Figure 1, page 13).
2. **Sample Wells -** add 20 µl of each sample to at least two wells.
3. Add 100 µl of Assay Buffer, 20 µl of Cofactor Mixture, and 20 µl 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 30 minutes at room temperature and then read using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. Fluorescence is stable 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 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 D-lactate from Table 1. See Figure 2, on page 18, for a typical standard curve.
4. Calculate the D-lactate concentration of the samples using the equation below.

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

*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 D-lactate values.

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

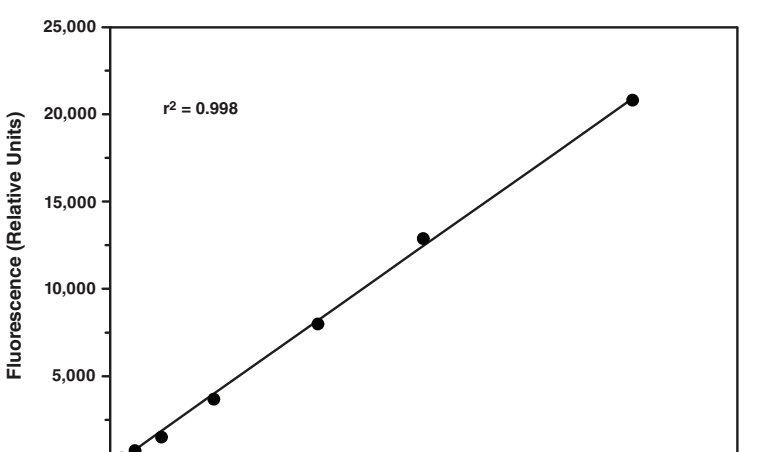

Figure 2. D-Lactate standard curve

Performance Characteristics

Precision:

When a series of eight deproteinated blood or eight deproteinated plasma mesurements were performed on the same day, the intra-assay coefficient of variation was 2.5% and 3.7%, 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 2.8% and 4.1%, respectively.

Assay Specificity:

To assess substrate specificity, the assay was performed with D-lactate replaced by structurally similar compounds. Compounds such as pyruvate, L-Lactate, and α-keto acids might interfere with the measurement of D-Lactate. Therefore, fluorescence signals generated by D-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 oxaloacetate, phosphenolpyruvate, and 2-oxobutyrate at 1 mM final concentration, or L-Lactate and pyruvate at a final concentration of 2 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 the sample using a lower dilution
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read
The fluorescence of the sample wells were higher than the last standard	Sample was too concentrated	Re-assay the sample using a higher dilution

References

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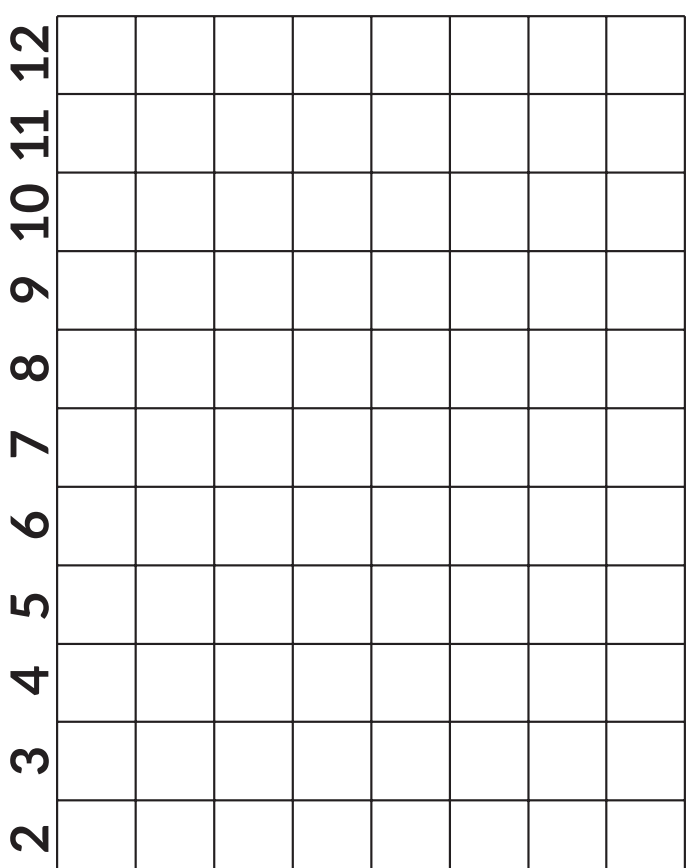
2. Flick, M.J. and Konieczny, S.F. Identification of putative mammalian D-lactate dehydrogenase enzymes. *Biochem. Biophys. Res. Commun.* **295(4)**, 910-916 (2002).
3. Bongaerts, G., Tolboom, J., Naber, T., *et al.* D-Lactic acidemia and aciduria in pediatric and adult patients with short bowel syndrome. *Clin. Chem.* **41(1)**, 107-110 (1995).
4. Poeze, M., Solberg, B.c., Greve, J.W., *et al.* Gastric $PgCO_2$ and $Pg\text{-}aCO_2$ gap are related to D-lactate and not to L-lactate levels in patients with septic shock. *Intensive Care Med.* **29(11)**, 1281-1285 (2003).
5. 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.

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