PractiChrom™ D-Lactate Assay Kit (PDLC-25)

Quantitative D-Lactate Determination Using PICOEXPLORER™

DESCRIPTION

D-LACTATE, or D-lactic acid, is generated by D-lactate dehydrogenase (LDH) under hypoxic or anaerobic conditions. D-lactic acid is produced in only minor quantities in animals and measuring for D-lactic acid in animal samples is a means to determine the presence of bacterial infection. Furthermore, since D-lactic acid is a specific indicator of bacterial fermentation, its measurement can be used to determine the freshness of milk, meat and fruit juices. Elevated levels of D-lactic acid in wine are an indication of lactic acid bacteria contamination.

BioAssay Systems' D-lactate assay is based on D-lactate dehydrogenase catalyzed oxidation of D-lactate in which the formed NADH reduces a chromogenic reagent. The intensity of product color is directly proportional to the D-lactate concentration in the sample.

KEY FEATURES

Sensitive and accurate. Detection limit of 0.05 mM (4.5 ppm, 0.45 mg/dL) and linearity to 1 mM (89 ppm, 8.9 mg/dL) D-lactate.

Convenient. Assay performed with portable PiCO Explorer device.

Cost efficient. No need for expensive plate readers.

APPLICATIONS

Direct Assays: D-lactate in beverage samples (e.g. red wine, beer, fruit juices, milk, etc) and biological samples (e.g. serum, plasma, etc).

KIT CONTENTS (25 TESTS)

Assay Buffer:	5 mL	**Standard:**	1.0 mL 20 mM D-Lactate
Enzyme A:	30 µL	**NAD/MTT:**	1.0 mL
Enzyme B:	120 µL	**ALT Enzyme:**	30 µL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Equilibrate all components to room temperature prior to assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation:

Red Wine. Dilute 20× in dH_2O by adding 10 µL sample to 190 µL dH_2O.

Beer. Dilute 10× in dH_2O by adding 10 µL sample to 90 µL dH_2O.

Juice. Dilute 2× in dH_2O by adding 20 µL sample to 20 µL dH_2O.

Serum & Plasma. Centrifuge to remove any particulates. No dilution is required.

Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 M HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 M NaOH. The neutralized supernatant is ready for assay (Dilution factor = 1.36).

Other colored samples or samples with high levels of D-lactate will require a dilution. For preparation protocols for other samples, please contact our technical support at info@bioassaysys.com

Procedure

1. Prepare 1 mM D-lactate Standard by mixing 10 µL of the provided 20 mM Standard and 190 µL dH_2O in an Eppendorf tube.
2. In separate PCR tubes, add 10 µL dH_2O and 10 µL 1 mM D-lactate Standard.

   Samples. Add 10 µL Sample to one PCR tube. For samples that are not colorless (e.g. red wine, most beer, most fruit juices, serum, plasma, etc), add 10 µL Sample to two separate tubes, one serving as the Sample tube and one as the Sample Blank tube.

   Reagent Preparation. Prepare sufficient Working Reagent (WR) for all dH_2O, Standard, and Sample tubes by mixing, for each tube: 40 µL Assay Buffer, 4 µL NAD/MTT, 0.5 µL Enzyme A, 0.5 µL Enzyme B, and 0.5 µL ALT Enzyme. For each Sample Blank tube, prepare Blank Working Reagent (BWR) *(No Enzyme A)*: 40 µL Assay Buffer, 4 µL NAD/MTT, 0.5 µL Enzyme B, and 0.5 µL ALT Enzyme.

   Then quickly add 40 µL WR to all dH_2O, Standard, and Sample tubes. To each Sample Blank tube, add 40 µL BWR. Close the tubes, briefly vortex or tap to mix. Tap tube on bench to settle liquid to the bottom of the tube if needed. Incubate for 15 min at room temperature in the dark.
3. Please refer to the PICOEXPLORER™ User's Manual for detailed instructions for operating the device.

   Download the PAS-110 application. Turn on Bluetooth.

   Push the Power button on the device. Then, open the app and tap the Connection Setting button and connect the device.

Measuring a Standard Curve (See pg 17-19 in User's Manual)

Return to the main menu and tap the Standard Curve button. Set the following:

LED Output: 10%

Unit: mM

RBG Selection: G

Tap the first Known Concentration Data Input Area box and input 0.0. Then, tap on the second box and input 1.0 (this represents the 0 and 1 mM D-lactate Standards). Place the dH_2O tube into the measurement chamber of the photo absorbance sensor. Tap the Known Concentration Measurement Input Area (the box below 0.00), and click Measure. Remove the tube, then place the 1 mM Standard into the measurement chamber. Tap the box below 1.00 and click Measure. Click Graph to view the standard curve.

Measuring Sample Concentrations

Return to the main menu and tap the Measure button. Edit the LED output, Units, and RBG selection as done above for the standard curve.

Place each Sample and Sample Blank tube into the measurement chamber of the photo absorbance sensor and tap measure.

CALCULATION

The "concentration" will be displayed on the PICOEXPLORER™ for each Sample and Sample Blank. To calculate the D-lactate concentration in the sample, subtract the Sample Blank concentration from the Sample concentration and multiply by the dilution factor used (e.g. 2, 10, 20, etc). If no sample blank was used, simply multiply the Sample concentration by the dilution factor.

$$[\text{D-Lactate}] = ([\text{Sample}] - [\text{Sample Blank}]) \times n \quad \text{(mM)}$$

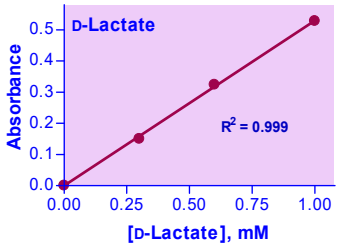
where [Sample] is the concentration of sample plus Enzyme A, [Sample Blank] is the concentration of sample *without* Enzyme A and *n* is the dilution factor.

Note: if the sample concentration says "Out of range" the sample is not within the linear range of the assay. If the color of the tube is yellow like the dH_2O tube, then the sample has low levels of D-lactate that cannot be detected by the assay. If the sample is very dark, dilute further in dH_2O and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM D-lactate equals 8.9 mg/dL, or 89 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, PCR tubes (e.g. Watson 137-211c 0.2 mL; or Cat# PCR-50 from BioAssay Systems), Eppendorf tubes (e.g. Phenix Cat# MAX-715, or Cat # EPP-50 from BioAssay Systems), and PICOEXPLORER™ (Cat # PICO001).



Standard Curve in water measured with PICOEXPLORER™