EnzyFluo™ D-Lactate Assay Kit (EFDLC-100)

Quantitative Fluorimetric Determination of D-Lactate

DESCRIPTION

Lactate is generated by lactate dehydrogenase (LDH) under hypoxic or anaerobic conditions. 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. D-Lactate is produced in only minor quantities in animals and measuring for D-lactate in animal samples is a means to determine the presence of bacterial infection.

Simple, direct and automation-ready procedures for measuring lactate concentration are very desirable. BioAssay Systems' EnzyFluo™ lactate assay kit is based on lactate dehydrogenase catalyzed oxidation of lactate, in which the formed NADH reduces a probe into a highly fluorescent product. The fluorescence intensity of this product, measured at $\lambda_{ex/em}$ = 530/585 nm, is proportional to the lactate concentration in the sample.

APPLICATIONS

Direct Assays: D-lactate in serum, plasma, urine, cell media samples and other biological samples.

KEY FEATURES

Sensitive and accurate. Detection limit of 1 µM and linearity up to 50 µM D-lactate in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the fluorescence after 60 min. Room temperature assay.

High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

KIT CONTENTS

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NAD Solution:	1 mL	**Enzyme B:**	120 µL
Probe:	750 µL	**Standard:**	1 mL

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION AND CONSIDERATIONS

The following substances interfere and should be avoided in sample preparation: EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%). Samples suspected of having endogenous L-LDH enzyme activity (e.g. serum, plasma, culture medium with FBS, etc.) should be deproteinated using a 10 kDa spin filter (e.g. Microcon YM-10). Deproteinated serum should be diluted 3 × with dH_2O. Samples containing higher than 50 µM pyruvate require an internal standard.

PROCEDURES

1. *Standard Curve*. Prepare 1000 µL 40 µM D-lactate Premix by mixing 2 µL 20 mM Standard and 998 µL distilled water. For cell culture samples, prepare 1000 µL 40 µM D-lactate Premix by mixing 2 µL 20 mM Standard and 998 µL culture medium without serum. Dilute standard as follows.

No	Premix + H_2O or Medium	D-Lactate (µM)
1	100 µL + 0 µL	40
2	60 µL + 40 µL	24
3	30 µL + 70 µL	12
4	0 µL + 100 µL	0

Transfer 50 µL standards into wells of a black, flat bottom 96-well plate.

Samples. Add 50 µL sample to two separate wells. Set up two reactions for each sample: one with added Enzyme A (Sample) and a No Enzyme A control (Sample Blank).

Samples requiring an internal standard, will need three separate reactions: 1) Sample plus Standard, 2) Sample alone and 3) Sample Blank. For the internal standard first prepare 400 µL 250 µM D-lactate standard by mixing 5 µL 20 mM Standard and 395 µL dH_2O. For the Sample plus Standard well, add 5 µL 250 µM D-lactate and 45 µL sample. For the Sample and Sample Blank wells, add 5 µL dH_2O and 45 µL sample.

2. Reagent Preparation. Spin the Enzyme tubes briefly before pipetting. For each Sample and Standard well, prepare Working Reagent by mixing 40 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 10 µL NAD and 5 µL Probe. Fresh reconstitution is recommended. For the Sample Blanks, the Working Reagent includes 40 µL Assay Buffer, 1 µL Enzyme B, 10 µL NAD and 5 µL Probe (**NO Enzyme A**).
3. Reaction. Add 50 µL Working Reagent per reaction well quickly. Tap plate to mix briefly and thoroughly. Incubate for 60 min at RT protected from light.
4. Read fluorescence $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

Plot the D-lactate Standard Curve and determine its slope. The D-lactate concentration of the sample is computed as follows:

$$[\text{D-Lactate}] = \frac{F_{SAMPLE} - F_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and Sample Blank respectively. Slope is the slope of the standard curve and *n* is the dilution factor (e.g. *n* = 3 for serum samples).

If an internal standard was needed, the sample D-lactate concentration is computed as follows:

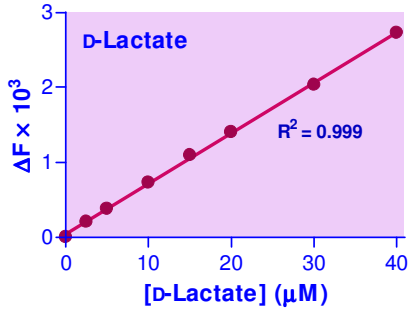
$$[\text{D-Lactate}] = \frac{F_{SAMPLE} - F_{BLANK}}{F_{STANDARD} - F_{SAMPLE}} \times 27.8 \quad (\mu M)$$

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and Sample Blank respectively and $F_{STANDARD}$ is the fluorescence intensity value of the Sample plus Standard.

Note: if the sample ΔF value is higher than the ΔF for 40 µM D-lactate standard or greater than the ΔF for the internal standard, dilute the sample in water and repeat the assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting (multi-channel) devices. Black, flat bottom 96-well plates and fluorescent plate reader capable of reading at $\lambda_{ex/em}$ = 530/585 nm.



Standard Curve in 96-well plate assay in water.

LITERATURE

1. Ewaschuk JB et al (2005). D-lactate in human and ruminant metabolism. J Nutr. 135(7):1619-25.
2. Mack DR (2004). D(-)-lactic acid-producing probiotics, D(-)-lactic acidosis and infants. Can J Gastroenterol. 18(11): 671-5.
3. Uribarri J et al (1998). D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. Medicine (Baltimore) 77(2):73-82.