**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 $\lambda_{\text{ex}} = 530/585$ nm, is proportional to the lactate concentration in the sample.

**APPLICATIONS**

**Direct Assays:** D-lactate in serum, plasma, urine, cell media 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 sample should be diluted 3 x with dH₂O. 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:

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + H₂O or Medium</th>
<th>D-Lactate (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µL + 0 µL</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>60 µL + 40 µL</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>30 µL + 70 µL</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>0 µL + 100 µL</td>
<td>0</td>
</tr>
</tbody>
</table>

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 dH₂O. 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 dH₂O 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.** $\lambda_{\text{ex}} = 530/585$ nm.

**CALCULATION**

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

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

where $F_{\text{SAMPLE}}$ and $F_{\text{BLANK}}$ 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:

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

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

**MATERIALS REQUIRED, BUT NOT PROVIDED**

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

**LITERATURE**