

**EnzyFluo™ L-lactate Assay Kit (EFLLC-100)**  
**Quantitative Fluorimetric Determination of L-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.

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 λ<sub>ex/em</sub> = 530/585 nm, is proportional to the lactate concentration in the sample.

**APPLICATIONS**

**Direct Assays:** L-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 L-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**

<b>Assay Buffer:</b>	10 mL	<b>Enzyme A:</b>	120 μL
<b>NAD Solution:</b>	1 mL	<b>Enzyme B:</b>	120 μL
<b>Probe:</b>	750 μL	<b>Standard:</b>	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%). Serum and plasma samples should be diluted at least 200× with dH<sub>2</sub>O. Samples containing higher than 100 μM pyruvate (final concentration after any dilutions) require an internal standard.

**PROCEDURES**

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

No	Premix + H <sub>2</sub> O or Medium	L-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 96-well plate.

**Samples.** Add 50 μL of each sample to separate wells of a black 96-well plate.

Samples requiring an internal standard, will need two separate reactions: 1) Sample plus Standard and 2) Sample alone. In addition, each plate will need a Water Blank (0 μM L-lactate) reaction. For the internal standard first prepare 400 μL 250 μM L-lactate standard by mixing 5 μL 20 mM Standard and 395 μL dH<sub>2</sub>O. For the Sample plus Standard well, add 5 μL 250 μM L-lactate and 45 μL sample. For the Sample wells, add 5 μL dH<sub>2</sub>O and 45 μL sample. For the Water Blank add 50 μL dH<sub>2</sub>O.

- 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.
- Reaction.** Add 50 μL Working Reagent per reaction well quickly. Tap plate to mix. Incubate for 60 min at RT protected from light.
- Read fluorescence** λ<sub>ex/em</sub> = 530/585 nm.

**CALCULATION**

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

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

where F<sub>SAMPLE</sub> and F<sub>BLANK</sub> are the fluorescence intensity values of the Sample and 0 μM L-lactate (Std 4) respectively. Slope is the slope of the standard curve and n is the dilution factor (e.g. n = 200 for serum samples).

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

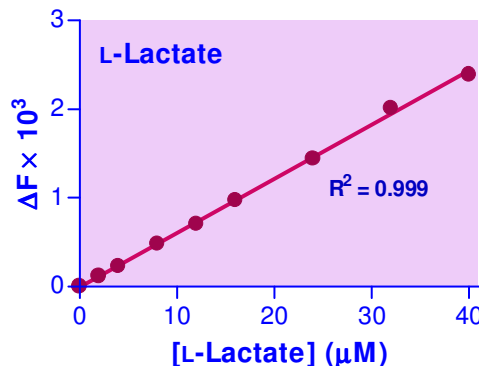
$$[L\text{-Lactate}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{F_{\text{STANDARD}} - F_{\text{SAMPLE}}} \times 27.8 \quad (\mu\text{M})$$

where F<sub>SAMPLE</sub> and F<sub>BLANK</sub> are the fluorescence intensity values of the Sample and Water Blank respectively and F<sub>STANDARD</sub> is the fluorescence intensity value of the Sample plus Standard.

Note: if the sample ΔF value is higher than the ΔF for 40 μM L-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 λ<sub>ex/em</sub> = 530/585 nm.



Standard Curve in 96-well plate assay in water.

**LITERATURE**

- Senadheera D et al (2009). Inactivation of VicK affects acid production and acid survival of Streptococcus mutans. J Bacteriol. 191(20):6415-24.
- Le Nihouannen D et al (2009). Ascorbic acid accelerates osteoclast formation and death. Bone 46(5):1336-43.
- Milovanova TN et al (2008). Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. Mol Cell Biol. 28(20):6248-61.