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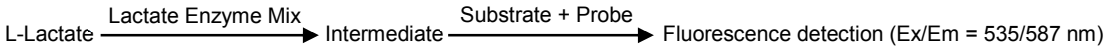
PicoProbe™ Lactate Fluorometric Assay Kit

rev. 2/13

(Catalog # K638-100; 100 assays; Store at -20°C)

I. Introduction:

Lactate ($CH_3CH(OH)COO^-$) plays an important role in many biological processes. Abnormally high concentrations of lactate have been related to diseases such as diabetes, lactic acidosis, etc. L(+)-Lactate is present in blood and is the major lactate stereoisomer formed in human intermediary metabolism. D-Lactate is also present but only at about 1-5% of L(+)-Lactate concentration. Lactate is a significant energy source for living organisms and can be used to generate cellular ATP. BioVision's PicoProbe™ Lactate Assay kit is suitable for measuring very low levels of L(+)-lactate in a variety of samples. In this assay, L(+)-lactate is specifically oxidized to form an intermediate that reacts with a colorless probe to generate fluorescence (Ex/Em = 535/587 nm), which is directly proportional to the amount of lactate. This simple, rapid & high-throughput suitable assay kit is the most sensitive Lactate Assay kit on the market. It can detect L(+)-lactate less than 0.2 µM in a variety of biological samples.



II. Application:

- Measurement of L(+)-lactate in various tissues/cells.
- Analysis of metabolism and cell signaling.
- Mechanistic study for diabetes.

III. Sample Type:

- Serum, Plasma etc.
- Animal tissues: Liver, muscle, heart etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	K638-100	Cap Code	Part Number
Lactate Assay Buffer	25 ml	WM	K638-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K638-100-2
Lactate Enzyme Mix (Lyophilized)	1 vial	Green	K638-100-3
Lactate Substrate Mix (Lyophilized)	1 vial	Red	K638-100-4
L(+)-Lactate Standard (100 mM)	100 µl	Yellow	K638-100-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat clear bottom. Black plates are preferred for fluorometric assays.
- Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Lactate Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **PicoProbe™ (in DMSO):** Ready to use as supplied. Briefly warm at 37°C to bring to room temperature before use. Store at -20°C.
- **Lactate Enzyme Mix**: Reconstitute with 220 µl Lactate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **Lactate Substrate Mix**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.

VIII. Lactate Assay Protocol:

1. **Sample Preparation:** Liquid samples can be measured directly. Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 100 µl cold Lactate Assay Buffer on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (1-10 µg) into a 96 well plate and adjust the volume to 50 µl with Lactate Assay Buffer.

   Notes:
   A. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
   B. NADH in samples will generate background. For samples having high NADH levels, a sample background control is required to subtract the background.
   C. Since proteins and various enzymes in samples may interfere with the assay, we recommend deproteinizing the samples using either perchloric acid/KOH protocol (BioVision Cat # K808-200) or using 10K spin column (BioVision Cat # 1997-25).

2. **Standard Curve Preparation:** Dilute Lactate Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Lactate Standard to 990 µl dH_2O, mix well. Dilute 1 mM Lactate Standard further to 25 µM (25 pmol/µl) by adding 10 µl of 1 mM Lactate Standard to 390 µl of dH_2O. Add 0, 2, 4, 6, 8 and 10 µl of the 25 µM Lactate Standard into series of wells in 96 well plate to generate 0, 50, 100, 150, 200, and 250 pmol/well Lactate Standards. Adjust volume to 50 µl/well with Lactate Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays (Standard & samples) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
Lactate Assay Buffer	44 µl	46 µl
PicoProbe™	2 µl	2 µl
Lactate Enzyme Mix	2 µl	----
Lactate Substrate Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard & test samples & 50 µl of Background Control Mix to background control well(s). Mix well.

4. **Measurement:** Incubate for 30 min. at room temperature, protected from light. Measure fluorescence at Ex/Em = 535/587 nm in a micro plate reader.
5. **Calculation:** Subtract 0 Lactate Standard reading from all readings. Plot the Lactate Standard curve. If sample background control reading is significantly high, subtract background control reading from sample reading. Apply corrected sample reading to the Lactate Standard Curve to get B pmol of Lactate amount in the samples.

Sample Lactate concentration (C) = B/V x Dilution Factor = pmol/µl = nmol/ml

Where: **B** = Amount of Lactate from the Standard Curve (pmol).
V = Sample volume used in the reaction well (µl).
Lactate molecular weight: 90.08 g/mol.

Lactate in samples can also be expressed in pmol/mg of protein or other desired method.

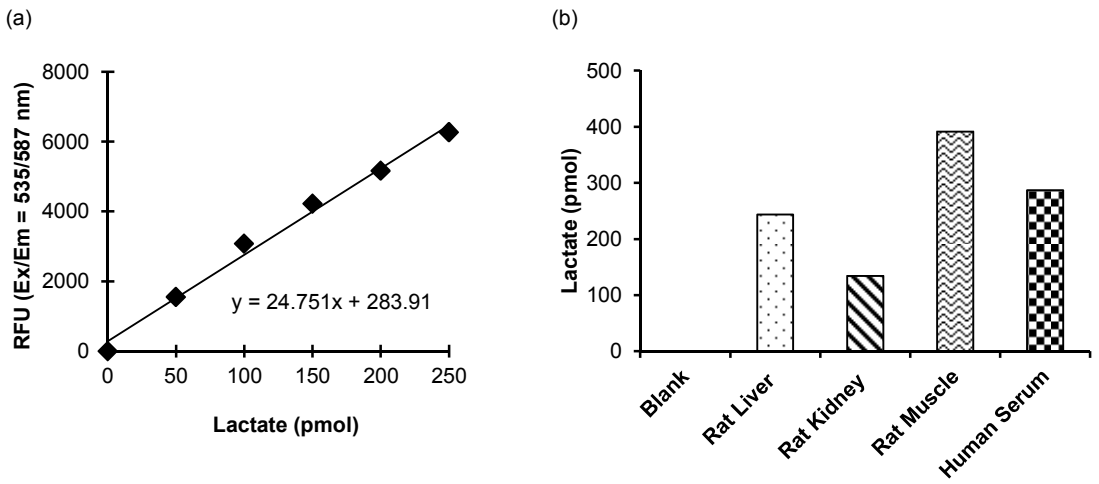


Figure: Lactate Standard Curve (a). Measurement of Lactate levels in rat liver (1.2 µg), kidney (0.7 µg) & muscle (0.45 µg) & in human serum (0.5 µl from 1:10 diluted serum) (b). Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

Lactate Assay Kit I & II
Lactate Dehydrogenase (LDH) Activity Assay Kit
D-Lactate Colorimetric Assay Kit
LDH Cytotoxicity Assay Kit II
Glucose Assay Kit I & II
Glucose and Sucrose Assay Kit
Glucose Uptake Colorimetric Assay Kit
Glucose Uptake Fluorometric Assay Kit
PicoProbe™ Glucose-6-Phosphate Assay Kit
Glucose-6-Phosphate Dehydrogenase Assay Kit
Glucose Dehydrogenase Activity Assay Kit
Maltose and Glucose Assay Kit
NAD/NADH Quantification Kit
NADP/NADPH Quantification Kit

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