ab169557

Picoprobe L-Lactate Assay Kit

Instructions for Use

For the sensitive and accurate measurement of L(+)-lactate in cells and tissues, plasma, serum and other biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 2a, Last Updated 8 June 2023

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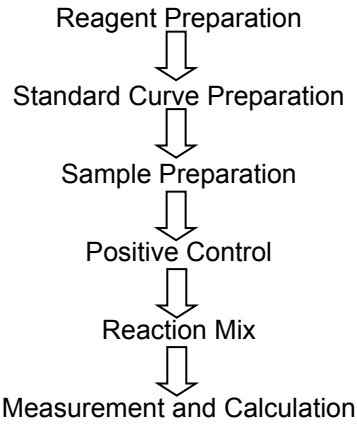
1. Overview

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.

Abcam's Picoprobe L-Lactate Assay Kit (ab169557) 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 and 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.

2. Protocol Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
Assay Buffer XII/Lactate Assay Buffer	25 mL	-20°C	-20°C
PicoProbe I/PicoProbe Probe (in DMSO)	0.4 mL	-20°C	-20°C
Enzyme Mix XV/Lactate Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Lactate Substrate Mix/Lactate Substrate Mix (lyophilized)	1 vial	-20°C	-20°C
L(+)-Lactate Standard/L(+)-Lactate Standard (100 mM)	100 µL	-20°C	-20°C

4. Storage

Store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm Assay Buffer XII/Lactate Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Additional Materials Required

- 96-well black plate with flat bottoms
- Multi-well spectrophotometer (ELISA reader)

6. Assay Protocol

A. Reagent Preparation

1. PicoProbe I/PicoProbe™ Probe:

Ready to use as supplied. Briefly warm at 37°C to bring to room temperature before use.

2. Enzyme Mix XV/Lactate Enzyme Mix:

Reconstitute with 220 µL Assay Buffer XII/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

3. 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.

B. L-Lactate Assay Protocol

1. **Lactate Standard Curve:**

   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 Assay Buffer XII/Lactate Assay Buffer.

2. **Sample preparation:**

   Liquid samples can be measured directly. Tissue (10 mg) or cells (1×10^6) should be rapidly homogenized with 100 µL cold Assay Buffer XII/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 Assay Buffer XII/Lactate Assay Buffer

   Notes: For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

   NADH in samples will generate background. For samples having high NADH levels, a sample background control is required to subtract the background.

   Since proteins and various enzymes in samples may interfere with the assay, we recommend deproteinizing the samples using either perchloric acid/KOH protocol or using 10K spin column.

3. Reaction Mix:

Mix enough reagents for the number of samples and standards to be performed. For each well, prepare a total 50 µL Reaction Mix containing:

	Reaction Mix	Background Control Mix
Assay Buffer	44 µL	46 µL
XII/Lactate Assay Buffer		
PicoProbe I/PicoProbe	2 µL	2 µL
Enzyme Mix	2 µL	—
XV/Lactate Enzyme Mix		
Lactate Substrate Mix	2 µL	2 µL

Add 50 µL of the Reaction Mix to each well containing the standard, test samples and background controls, mix well.

4. Measurement

Incubate for 30 minutes at room temperature, protected from light. Measure fluorescence at Ex/Em = 535/587 nm in a micro plate reader.

7. Data Analysis

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.

$$\textbf{Sample lactate concentration (C)} = \frac{\textbf{B}}{\textbf{V}} \times \textbf{Dilution Factor} = \textbf{pmol/µL = nmol/mL}$$

Where:

B is the amount of Lactate from the standard curve (pmol).

V is the sample volume used in the reaction well (mL).

Lactate molecular weight: 90.08 g/mol.

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

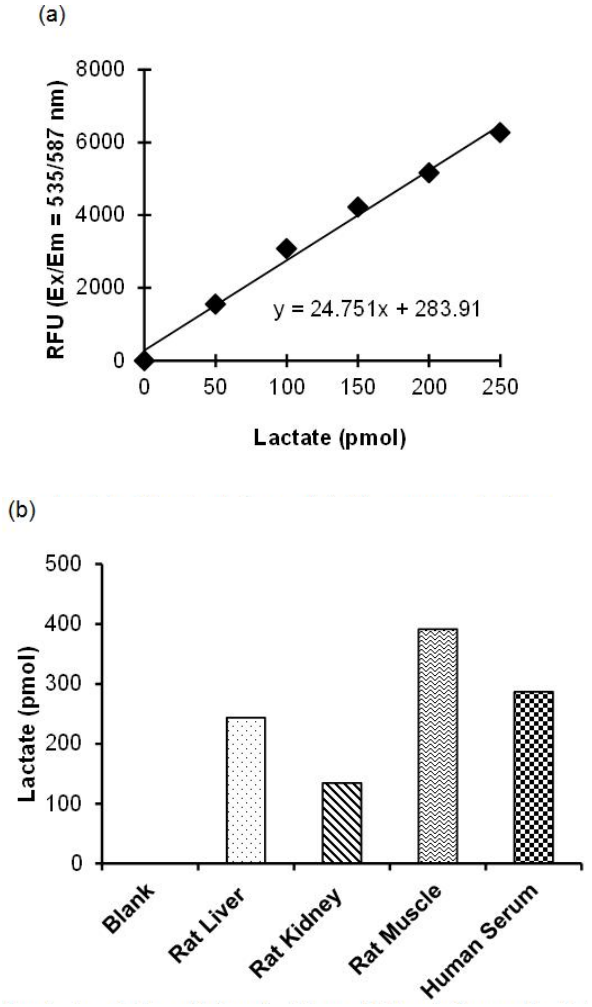


Figure 1: (a) Lactate standard curve (b) Measurement of Lactate levels in rat liver (1.2 µg), kidney (0.7 µg), and muscle (0.45 µg) and in human serum (0.5 µL from 1:10 diluted serum). Assays were performed following kit protocol.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)

Problem	Reason	Solution
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix

Problem	Reason	Solution
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.co.jp/contactus (Japan)