Product Information

Lactate Fluorometric Assay Kit

I. Kit Contents:

Components	K2140-100 100 assays	Cap Color	Part Number
Lactate Assay Buffer	25 ml	WM	K2140-C-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K2140-C-2
Lactate Enzyme Mix (Lyophilized)	1 vial	Green	K2140-C-3
Lactate Substrate Mix (Lyophilized)	1 vial	Red	K2140-C-4
L(+)-Lactate Standard (100 mM)	100 µl	Yellow	K2140-C-5

II. Introduction:

Lactate is an important energy source for living organisms and can generate cellular ATP. Lactate is chiral: L(+)-Lactate and D(-)-Lactate. L(+)-Lactate exists in blood and is constantly produced from pyruvate by lactate dehydrogenase (LDH) in human intermediary metabolism. D(-)-Lactate is present only at about 1-5% of the concentration of L(+)-Lactate. Abnormal high concentration of lactate is related to diseases such as lactate acidosis and diabetes.

The Lactate Fluorometric Assay Kit provides a highly sensitive, simple and convenient way for detection of very low levels of L(+)-lactate in various biological samples based on fluorometric method. In the assay, L(+)-lactate is specifically oxidized to produce an intermediate that reacts with a colorless probe to yield fluorescence (Ex/Em = 535/587 nm), which is directly proportional to the amount of lactate. The kit is suited for high-throughput assay and can detect less than 0.2 µM L(+)-lactate in various biological samples.

III. Application:

Measurement of L(+)-lactate in various tissues/cells.

Analysis of metabolism and cell signaling.

Mechanistic study for diabetes.

IV. Sample Type:

Serum, Plasma etc.

Animal tissues: Liver, muscle, heart etc.

Cell culture: Adherent or suspension cells.

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℃, 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℃ to bring to room temperature before use. Store at -20℃.

Lactate Enzyme Mix: Reconstitute with 220 µl Lactate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20℃. 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℃. 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 or using 10K spin column.

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.

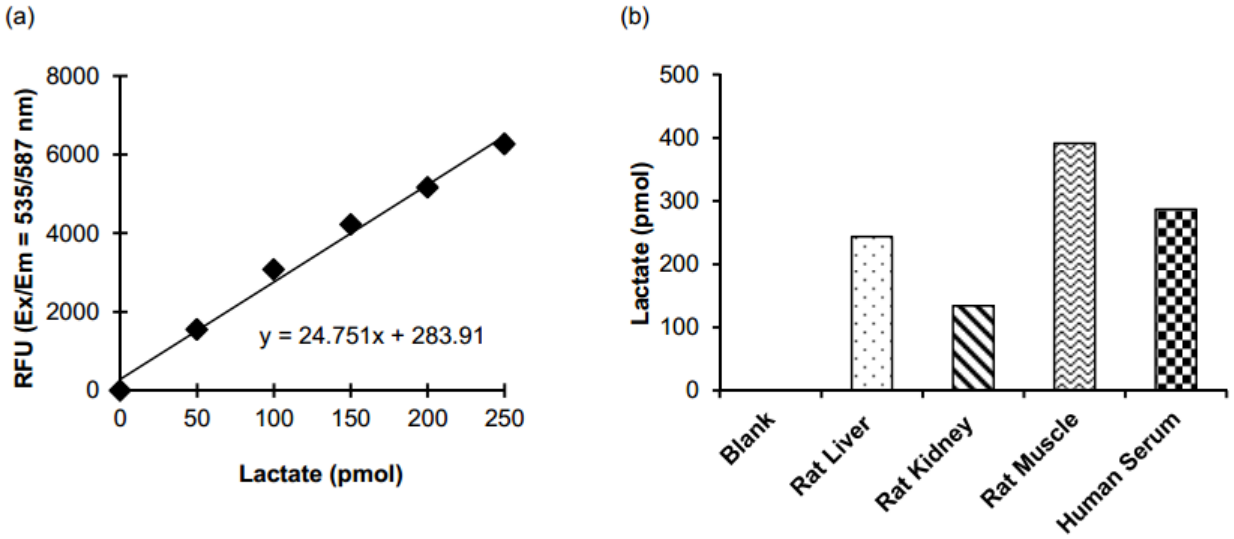

Figure: Lactate Standard Curve (a). Measurement of Lactate levels in rat liver (1.2 µg), kidney (0.7 µg) & muscle (0.45 µg) & in human serum (0.5 µl from 1:10 diluted serum) (b). Assays were performed following kit protocol.

Frequently Asked Questions:

1. The fluorescence counts are very low (less than 100) in the standards. Samples show higher counts (100 - 300) but when the amount of samples is doubled, the fluorescence intensity is not doubled. What could have gone wrong?

The assay buffer and the probe need to be warmed to RT or 37℃ before the assay. This is essential for the reaction to proceed at its normal pace. If either components are not warmed up enough, the reaction is slow and after 30 mins of incubation, you could get low readings and readings that are not linearly correlated. There is a lag time in this case when the enzyme activity ramps up. You could incubate longer, but that would not result in the highest possible readings. Warming up these two components, mixing them well and spinning down to make sure nothing is stuck to the cap and then proceeding with the assay should help. Adding more samples might have led to saturation and hence RFU values do not double upon adding twice the amount of sample.

2. Will carrying out the reaction at 37℃ help instead of RT?

Our recommended incubation temperature is RT (25℃).It is not recommended to increasing it to 37℃. The enzymes in the assay might show different kinetics at 37℃ than what was used to develop and optimize the assay. Some enzymes slow down at sub-optimal temperatures.

3. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

4. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. It is recommended to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

5. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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