Product Information

Lactate Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2092-100 100 assays	Cap Color	Part Number
Lactate Assay Buffer	25 ml	WM	K2092-C-1
Lactate Probe (in DMSO, anhydrous)	0.2 ml	Red	K2092-C-2
Lactate Enzyme Mix	1 vial	Green	K2092-C-3
L(+)-Lactate Standard (100 nmol/µl)	100 µl	Yellow	K2092-C-4

II. Introduction:

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 Colorimetric/Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of lactate in various biological samples (blood circulation, cells, culture mediums, fermentation mediums, etc.) based on colorimetric and fluorometric method. In the assay, lactate specifically reacts with lactate enzyme mix to yield a product, which interacts with lactate probe to produce fluorescence (at Ex/Em = 535/587 nm) and color (570 nm). The assay can be performed without pretreatment or purification of samples. The kit can detect 0.001 - 10 mM lactate samples.

III. Application:

Measurement of Lactate in various biological samples.

Analysis of metabolism in various cells.

Diabetes research.

IV. Sample Type:

Culture medium.

Fermentation medium.

Blood.

Cells.

V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at -20℃, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

Lactate Assay Buffer: Warm to room temperature before use. Store at -20℃ or 4℃.

Lactate Probe: Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at -20℃, protected from light. Use within two months.

Lactate Enzyme Mix: Dissolve in 220 µl Lactate Assay Buffer. Pipet up and down to completely dissolve. Store at -20℃. Use within two months.

VIII. Lactate Assay Protocol:

1. Sample Preparation: Add 2 - 50 µl test samples to a 96-well plate. Adjust the volume to 50 µl/well with Lactate Assay Buffer. If using serum sample, serum (0.5-10 µl/assay, serum contains ~ 0.6 nmol/µl lactate) can be directly diluted in the Lactate Assay Buffer.

Note:

a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

b. For samples having high background, prepare parallel well(s) containing same amount of sample as in the test well as background control.

c. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Lactate in the test samples, we recommend spiking samples with a known amount of Standard (4 nmol).

d. Complete medium containing FBS should be deproteinized due to high LDH content. Lactate Dehydrogenase (LDH) will degrade lactate. Therefore, samples containing LDH (such as culture medium containing FBS or tissue lysate) should be kept at -80℃ for storage, or filter samples through 10 kDa molecular weight spin filter.

2. Standard Curve Preparation: For the colorimetric assay, dilute the Lactate Standard (MW 90.08) to 1 nmol/µl by adding 10 µl of the 100 nmol/µl Lactate Standard to 990 µl of Lactate Assay Buffer, mix well. Add 0, 2, 4, 6, 8 & 10 µl into a series of wells. Adjust the volume to 50 µl/well with Lactate Assay Buffer to generate 0, 2, 4, 6, 8 & 10 nmol/well of the L(+)-Lactate Standard. For fluorometric assay, dilute the Lactate Standard to 0.01 nmol/µl by adding 10 µl of the 1 nmol/µl Lactate Standard to 990 µl of Lactate Assay Buffer, mix well. Add 0, 2, 4, 6, 8 & 10 µl of 0.01 nmol/µl Lactate Standard into a series of wells. Adjust the volume to 50 µl/well with Lactate Assay Buffer to generate 0, 20, 40, 60, 80 & 100 pmol/well of the Lactate Standard.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µl Reaction Mix containing the following components.

	Reaction Mix	Background Control Mix
Lactate Assay Buffer	46 µl	48 µl
Lactate Probe	2 µl	2 µl
Lactate Enzyme Mix	2 µl	---

Mix well. Add 50 µl of the Reaction Mix to each well containing the Lactate Standards & test samples and mix well.

Note:

For samples having high background, add 50 µl of Background Control Mix to sample background control well(s).

a. The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading.

4. Measurement: Incubate the reaction for 30 min. at room temperature, protected from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/590 nm) in a microplate reader.

5. Calculation: Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Lactate Standard Curve. For unspiked samples, apply the corrected OD to the Lactate Standard Curve to get B nmol of Lactate in the sample well.

Sample Lactate concentration (C) = B/V X D nmol/µl or mM.

Where: B is the amount of Lactate in the sample well (nmol).

V is the sample volume added into the reaction well (µl).

D is the sample dilution factor.

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Lactate amount in sample well (B) = (OD sample (corrected))/ (OD $_{\text{sample + Lactate Std(corrected)}}$× OD $_{\text{sample (corrected)}}$))* Lactate Spike (nmol)

Lactic acid molecular weight: 90.08.

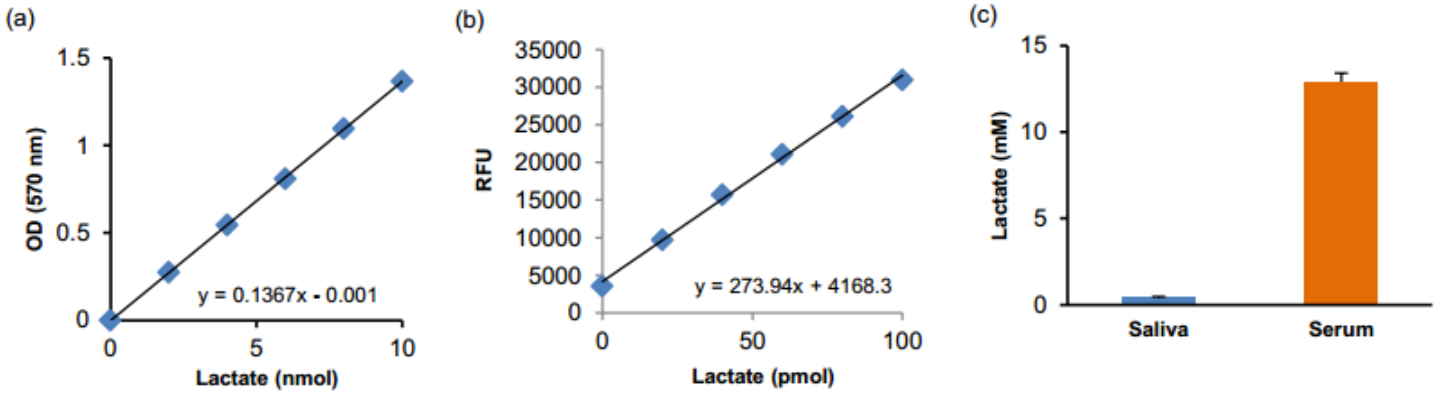

Figure: Lactate Standard Curve: colorimetric (a), fluorometric (b). c.) Quantitation of lactate in human saliva & serum. Saliva was centrifuged at 10000 x g for 10 min. at 4°C. 3 µl of supernatant was spiked with a known amount of lactate (4 nmol) as internal Standard. Serum was diluted 20-fold and 3 µl was assayed. Calculated concentrations (mg/dl): Saliva: 0.42 ± 0.05; Serum: 12.9 ± 0.5. Assays were performed according to the kit protocol.

Frequently Asked Questions:

1. Is there difference between incubation of 37℃ and Room Temp?

The difference between room temperature and 37℃ is not significant enough to affect the results. The reaction is slower at RT.

2. What is the explanation for seeing lower readings at higher conc. of sample?

Higher concentration of Lactate does have inhibitory effect that leads to lower readings. So, smaller amount of sample is recommended to generate values that fit in the linear part of the standard curve. When there is lactate overdose, the reaction will appear dark pink and then turns brown.

3. What kind of medium should be used with this assay?

Medium devoid of Lactate and/ or pyruvate should be used. Medium containing FBS should be deproteinized to remove LDH which can degrade Lactate.

4. Is it essential to deproteinize samples for this assay?

Yes, it is highly recommended to deproteinize samples/medium to remove enzymes such as LDH which can quickly degrade Lactate.

5. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples (-80℃) can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods. For cell lysates/tissue homogenates or cell culture media, storing after deproteinizing is recommended.

6. How many cells should be grown per well so that the medium can then be assayed for lactate?

There are several variables: 1) Cell type and the metabolic sate of the cell 2) Medium glucose level 3) lactate/glucose ratio in the media. Since cell types vary, the number of cells used depends on the cell type and experimental data needs to be generated to predict the level of lactate Vs. time. As time passes, cells will be consuming glucose and produce lactate. As the level of glucose drops and the cell starve, they start consuming lactate. So, the level of lactates goes up with time and eventually it starts decreasing.

8. Can this assay be normalized with respect to protein concentration?

Since deproteinizing is important for preserving the lactate in the sample, it is difficult to normalize lactate concentration with protein in the sample. However, sample can be added to the deproteinizing spin filters based on total protein.

9. Can this assay be automated and used with 384-well plates?

Although we have optimized the volumes of reagents based on tests with a 96-well plate format, this assay can be automated and used with 384 well plates. It will be essential to proportionately scale down the reagents.

10. Will the phenol red in the media affect the assay readout?

Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

11. Does this asay detect D-lactate as well?

No, this assay is specific for L-lactate. If there is a substantial amount of D-lactate in the sample, a parallel background control can be run to exclude any possibility of interference.

12. Can this kit be measured using 530 nm filter?

It is always recommended to use the exact recommended wavelength for the most accurate 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 570 nm.

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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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