Lactate Colorimetric Assay Kit-384 Well Format (#BN01112)

(Catalog #BN01112; 384 assays; Store at -20°C)

I. Introduction:

Abnormal high concentration of lactate in blood has been related to many important diseases such as diabetes and lactate acidosis. Elevated Lactate concentration in blood is also associated with shock, post cardiac arrest, tissue ischemia, thiamine deficiency, liver disorder, mitochondrial diseases and many more. Therefore detection and quantitation of lactate in from blood is a diagnostic key for several diseases. Assay Genie's Lactate Colorimetric Assay Kit uses an enzyme mix which reacts with L-Lactate specifically. D-Lactate does not react with the enzyme mix. The enzymatic reaction with L-lactate produces a product that generates a color (OD= 590 nm) when reacts with the Lactate probe. The formed product is directly proportional to the amount of L-Lactate present in the sample. The method is quantitative, rapid, simple, sensitive, and designed for high throughput format. The kit provides a convenient means for detecting 0.5 to 5 mM Lactate in biological samples such as in blood, blood circulation, in cells, in culture media, in fermentation media etc. in 384 HTP format.

II. Application:

- Measurement of L-Lactate in various biological samples
- Analysis of Lactate metabolism in various cells
- Research on diabetes, hemorrhagic shock, liver failure and other diseases

III. Sample Type:

- Culture medium
- Fermentation medium
- Blood, Serum, Plasma

IV. Kit Contents:

Components	BN01112	Cap Code	Part Number
Lactate Assay Buffer	25 ml	WM	BN01112-1
Lactate Probe (in DMSO, anhydrous)	800 µl	Red	BN01112-2A
Lactate Enzyme Mix (lyophilized)	1 vial	Green	BN01112-3
Lactate Standard (100 mM)	100 µl	Yellow	BN01112-4

V. User Supplied Reagents and Equipment:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

VI. Storage and Handling:

Store kit at -20°C, 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°C or 4°C.
- **Lactate Probe:** Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at -20°C, protected from light. Use within two months. Aliquot to avoid multiple freeze thaws.
- **Lactate Enzyme Mix**: Dissolve in 220 µl Lactate Assay Buffer. Pipet up and down to completely dissolve. Store at -20° C. Use within two months. When using, always keep in ice.

VIII. Lactate Assay Protocol:

1. **Sample Preparation:** Add 1-12.5 µl test sample to a 384-well plate. Adjust the volume to 12.5 µl/well with Lactate Assay Buffer. If using serum sample, use 1 µl serum/ well. Normal range of lactate in serum is 0.5 to 2.4 mM.

   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. Complete medium containing FBS should be deproteinized due to high Lactate Dehydrogenase (LDH) content. LDH will degrade lactate. Therefore, samples containing LDH (such as culture medium containing FBS, tissue lysate or serum) should be kept at -80°C for storage so that LDH remains inactive, or filter samples through 10 kDa molecular weight spin filter (Cat. # 1997) to remove the proteins from the sample. When working, always keep the samples on ice.

   d. Instrument reader settings must be adjusted according to the chosen 384-well plate. (The right dimension of the used 384-well plate may be available in the manual provided by the plate-manufacturer).

2. **Standard Curve Preparation:** Dilute the Lactate Standard (MW: 90.08 g/mol) to 0.5 mM by adding 5 µl of the 100 mM Lactate Standard to 995 µl of Lactate Assay Buffer, mix well. Add 0, 2, 4, 6, 8 & 10 µl into a series of wells to generate 0, 1, 2, 3, 4 & 5 nmol/well of the Lactate Standard. Adjust the volume to 12.5 µl/well with Lactate Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 12.5 µl Reaction Mix containing the following components.

	Reaction Mix	*Background Control Mix
Lactate Assay Buffer	10.0 µl	10.5 µl
Lactate Enzyme Mix	0.5 µl	--
Probe	2.0 µl	2.0 µl

Mix well. Add 12.5 µl of the Reaction Mix to each well containing the Lactate Standards & test samples and mix well.

Note:

* For samples having high background, add 12.5 µl of Background Control Mix to sample background control well(s).

4. **Measurement:** Incubate the reaction for 30 min. at room temperature, protected from light. Measure absorbance (OD: 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. Apply the corrected OD to the Lactate Standard Curve to get B nmol of Lactate in the sample well.

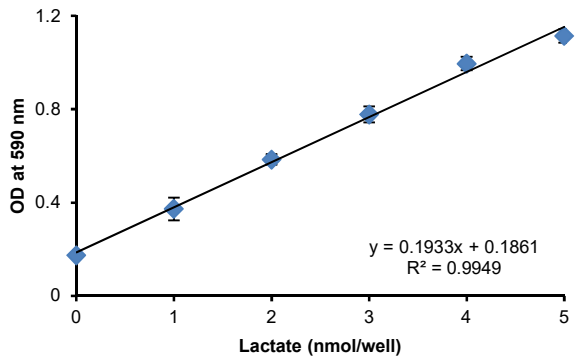
Sample Lactate concentration (C) = B/V X D nmol/µl or mM

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

Lactic acid molecular weight: 90.08 g/mol.

1 mM= 9.08 mg/dl

(a)



(b)

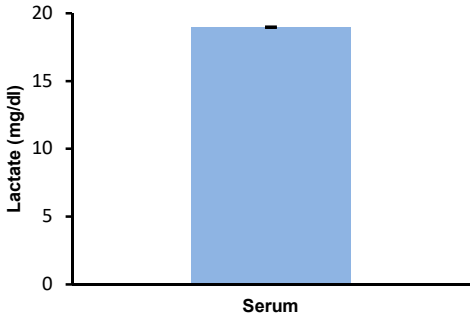


Figure: (a) Lactate Standard Curve. (b) Quantitation of L-Lactate in human serum. Serum sample was deproteinized using a 10 kDa Spin Column (10000xg, 10 min, 4°C). Undiluted deproteinized serum (1 ul) was assayed according to the kit protocol. Calculated concentrations (mg/dl): Serum: 18.96 ± 0.054. Assay was performed according to the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.