Lactate Colorimetric/Fluorometric Assay Kit (BN00831)

(Catalog # BN00831; 100 assays; Store at -20°C)

I. Introduction:

Abnormal high concentration of lactate has been related to disease states such as diabetes and lactate acidosis, etc. L(+)-Lactate is the major stereo-isomer of lactate formed in human intermediary metabolism and is present in blood. D(-)-Lactate is also present but only at about 1-5% of the concentration of L(+)-Lactate. In the Lactate Assay Kit, lactate specifically reacts with an enzyme mix to generate a product, which interacts with lactate probe to produce color (OD 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit provides a convenient means for detecting L(+)-Lactate in biological samples such as in blood circulation, in cells, in culture mediums, in fermentation mediums, etc. There is no need of pretreatment or purification of samples. The kit can detect 0.001-10 mM of Lactate in various samples. We recommend to use clear 96 well plate for both colorimetric & fluorometric assay.

II. Application:

- Measurement of Lactate in various biological samples
- Analysis of metabolism in various cells
- Diabetes research

III. Sample Type:

- Culture medium
- Fermentation medium
- Blood
- Cells

IV. Kit Contents:

Components	BN00831	Cap Code	Part Number
Lactate Assay Buffer	25 ml	WM	BN00831-1
Lactate Probe (in DMSO, anhydrous)	200 µl	Red	BN00831-2A
Lactate Enzyme Mix	lyophilized	Green	BN00831-3
L(+)-Lactate Standard (100 nmol/µl)	100 µl	Yellow	BN00831-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

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.
- **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.

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°C 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 Enzyme Mix	2 µl	--
Probe	2 µl	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) = $\left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + Lactate Std(corrected)}})\text{-}(\mathrm{OD}_{\text{sample(corrected)}})}\right)$ $*\ Lactate\ Spike\ (\mathrm{nmol})$

Lactic acid molecular weight: 90.08.

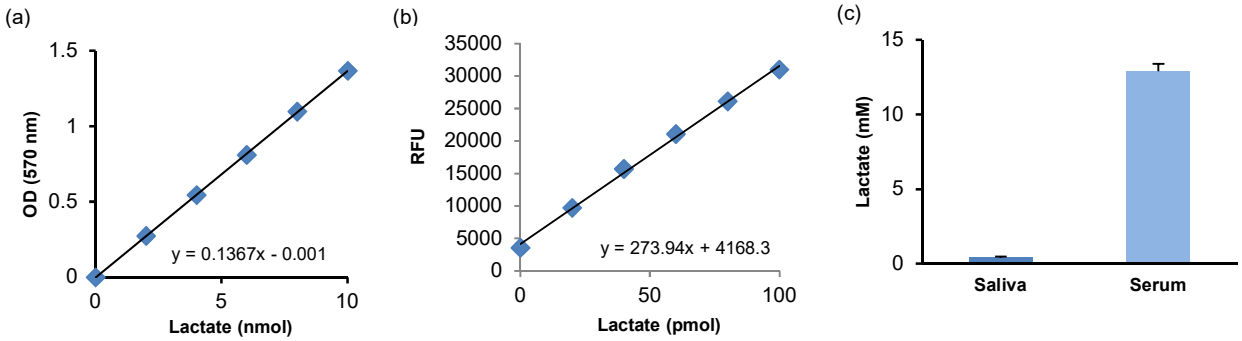

Figure: Lactate Standard Curve: colorimetric (a), fluorometric (b). c.) Quantitation of lactate in human saliva & serum. Saliva was centrifuged at 10000 x g for 10 min. at 4°C. 3 µl of supernatant was spiked with a known amount of lactate (4 nmol) as internal Standard. Serum was diluted 20-fold and 3 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.

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