

Lactate Assay Kit II

(Catalog #K627-100; 100 assays; Store kit at -20°C)

I. Introduction:

Lactate (CH₃CH(OH)COO⁻) plays important roles in many biological processes. Abnormally high concentrations of lactate have been related to disease states such as diabetes and lactic acidosis, etc. L(+)-Lactate is the major lactate stereoisomer 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 is oxidized by lactate dehydrogenase to generate a product which interacts with a probe to produce a color (λ_{max} = 450 nm). The kit detects L(+)-Lactate in biological samples such as in serum or plasma, cells, culture and fermentation media. There is no need for pretreatment or purification of samples. It detects 0.02 mM – 10 mM lactate in various samples.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Lactate Assay Buffer	25 ml	WM	K627-100-1
Lactate Enzyme Mix	lyophilized	Green	K627-100-2
Lactate Substrate Mix	lyophilized	Red	K627-100-3
L(+)-Lactate Standard (100 mM)	100 μ l	Yellow	K627-100-4

III. Reagent Preparation and Storage Conditions:

Lactate Enzyme Mix: Dissolve in 0.22 ml Lactate Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20°C. Use within two months.

Lactate Substrate Mix: Reconstitute with 0.22 ml of Lactate Assay Buffer and mix thoroughly. The solution is stable for 2 months at 4°C.

IV. Lactate Assay Protocol:

- Standard Curve Preparations:** Dilute the Lactate Standard (MW 90.08) to 1 mM by adding 10 μ l of the Lactate Standard to 990 μ l of Lactate Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 μ l into each well individually. Adjust volume to 50 μ l/well with Lactate Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the L(+)-Lactate Standard.
- Sample Preparation:** Prepare test samples at 50 μ l/well with Lactate Assay Buffer in a 96-well plate. For serum samples, 0.5-10 μ l serum can be directly tested (regular serum contains ~0.6 nmol/ μ l lactate). We suggest using several doses of your sample to ensure the readings are within the standard curve range.

Note: (1) Tissue or cells can be homogenized in the assay buffer. Centrifuge to remove the insoluble materials. The soluble fraction may be assayed directly.

(2) NADH or NADPH from cell or tissue extracts generates background for the lactate assay. To remove the NADH or NADPH background, same amount of sample can be tested in the absence of Lactate Enzyme Mix. Then the background readings can be subtracted from the lactate reading.

(3) Endogenous Lactate Dehydrogenase (LDH) may degrade lactate. Samples containing LDH (such as culture medium or tissue lysate) should be kept at -80°C for storage, or filtered through a 10Kd mw spin filter (BioVision, Cat 10kc-20) to remove all proteins.

- Reaction Mix Preparation:** Mix sufficient reagent for the number of assays performed. For each well, prepare a total 50 μ l Reaction Mix containing the following components. Mix well before use:

46 μ l Lactate Assay Buffer
2 μ l Lactate Substrate Mix
2 μ l Lactate Enzyme Mix

- Add 50 μ l of the Reaction Mix to each well containing the Lactate Standard or test samples, mix well.
- Incubate the reaction for 30 minutes at room temperature.
- Measure O.D. 450nm in a microplate reader. The color is stable for at least 4 hours.
- Calculation:** Correct background by subtracting the value derived from the 0 lactate control from all standard and sample readings (Note: Background can be significant and must be subtracted from all standard and sample readings). Plot a standard curve of nmol/well vs. OD_{450nm}. Apply the sample readings to the standard curve. Calculate the lactate concentrations of the test samples:

$$C = La/Sv \text{ (nmol}/\mu\text{l or mM)}$$

Where: La is the lactic acid amount (nmol) of your sample from standard curve.
Sv is the sample volume (μ l) added into the well.

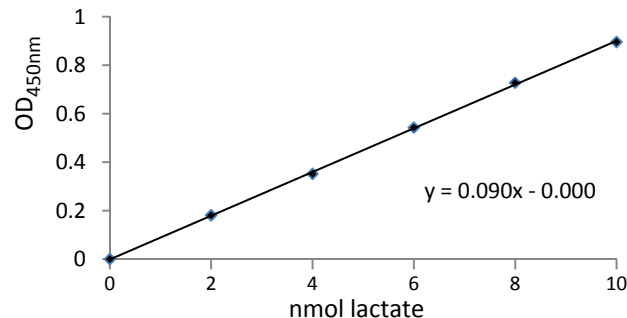


Fig: Lactate Standard Curve. The assay is performed follow the kit protocol.

V. Related Products:

Cholesterol Assay Kit
Glutathione Assay Kit
Glucose Assay Kit
Cell Proliferation Assay
Cytotoxicity Assay
Apoptosis Assay Related Products
Creatinine Assay Kit
Pyruvate Assay Kit
Lactate Fluorometric/Colorimetric Assay Kits