

Lactate Assay Kit

(Catalog #K607-100; 100 assays; Store kit 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 a enzyme mix to generate a product, which interacts with lactate probe to produce color (at $\lambda = 570$ nm) and fluorescence (at 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 various Lactate samples.

II. Kit Contents:

Components	100 assays	Cap color	Part Number
Lactate Assay Buffer	25 ml	WM	K607-100-1
Lactate Probe	1 Vial	Red	K607-100-2
DMSO (anhydrous)	400 μ l	Brown	K607-100-3
Lactate Enzyme Mix	1 Vial	Green	K607-100-4
L(+)-Lactate Standard (100 nmol/ μ l)	100 μ l	Yellow	K607-100-5

III. Reagent Preparation and Storage Conditions:

Lactate Probe: Dissolve the lactate probe with 220 μ l of dried DMSO (provided) before use. Mix, store at -20C, protect from light and moisture. Use within two months.

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

IV. Lactate Assay Protocol:

- Standard Curve Preparations:** For the colorimetric assay, dilute the Lactate Standard (MW 90.08) to 1 nmol/ μ l 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.

For fluorometric assay, dilute the Lactate Standard to 0.1 nmol/ μ l by adding 10 μ l of the Lactate Acid to 990 μ l of Lactate Assay Buffer, mix well. Then take 20 μ l into 180 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, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Lactate Standard.

- Sample Preparations:** Prepare test samples in 50 μ l/well with Lactate Assay Buffer in a 96-well plate. If using serum sample, serum (0.5-10 μ l/assay, serum contains ~0.6 nmol/ μ l lactate) can be directly diluted in the Lactate Assay Buffer. We suggest using several doses of your sample to ensure the readings are within standard curve range.

Note: Lactate Dehydrogenase (LDH) may degrade lactate. Therefore, the samples contain LDH (such as culture medium or tissue lysate) should be kept -80C for storage, or filter samples through 10 Kd molecular weight spin filter (BioVision, Cat 1997).

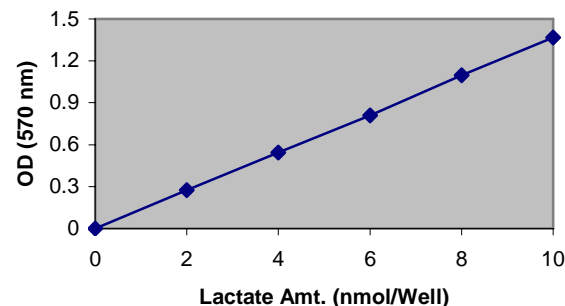
- Reaction Mix Preparation:** Mix enough reagent for the number of assays performed: For each well, prepare a total 50 μ l Reaction Mix containing the following components. Mix well before use.

- 46 μ l Lactate Assay Buffer
- 2 μ l Probe
- 2 μ l 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, protect from light.
- Measure O.D. 570 nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader. If the background is too high in the fluorometric assay, 1/10 volume of probe may be used, which will decrease the background significantly.
- Correct background by subtracting the value derived from the 0 lactate control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot standard curve nmol/well ~ OD570nm readings. Then apply the sample readings to the standard curve. Calculate the lactate concentrations of the test samples: $C = La/Sv$ (nmol/ μ l or mM)

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

Lactate acid molecular weight: 90.08. Lactate acid concentration in your sample can be expressed by the way of your choice.



V. Related Products:

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| Cholesterol Assay Kit | Glycogen/Starch Assay Kit |
| Glutathione Assay Kit | GST Assay Kit |
| Glucose Assay Kit | Triglycerides/Fatty Acid Assay Kit |
| Cell Proliferation Assay Kit | Ascorbate Assay Kit |
| Cytotoxicity Assay | Pyruvate Assay Kit |
| CETP Activity Assay Kit | NADH/NADPH Assay Kit |