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 λ = 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 (in DMSO, anhydrous)	200 µl	Red	K607-100-2A
Lactate Enzyme Mix	lyophilized	Green	K607-100-4
L(+)-Lactate Standard (100 nmol/µl)	100 µl	Yellow	K607-100-5

III. Reagent Preparation and Storage Conditions:

Lactate Probe: Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at –20°C, protect 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.

IV. Lactate Assay Protocol:

1. **Standard Curve Preparations:** For the colorimetric assay, dilute the Lactate Standard (MW 90.08) to 1 nmol/µl by adding 10 µl of the Lactate Standard to 990 µl of Lactate Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust 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 0.1 nmol/µl by adding 10 µl of the Lactate to 990 µl of Lactate Assay Buffer, mix well. Then take 20 µl into 180 µl of Lactate Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Lactate Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Lactate Standard.

2. **Sample Preparations:** Prepare test samples in 50 µl/well with Lactate Assay Buffer in a 96-well plate. 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:** Complete medium containing FBS should be deproteinized (see below) due to high LDH content. We suggest using several doses of your sample to ensure the readings are within standard curve range.

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

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

   46 µl Lactate Assay Buffer
   2 µl Probe
   2 µl Enzyme Mix

4. Add 50 µl of the Reaction Mix to each well containing the Lactate Standard or test samples, mix well.

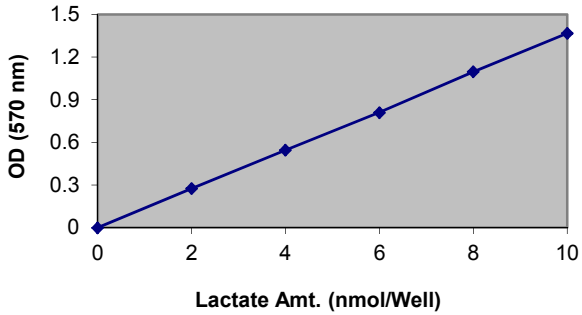
5. Incubate the reaction for 30 minutes at room temperature, protect from light.

6. Measure OD at 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.

7. 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 ~ OD_{570nm} readings. Apply the sample readings to the standard curve. Calculate the lactate concentrations of the test samples:

   C = La/Sv (nmol/µl or mM)

   Where: La is the lactate acid amount (nmol) of your sample from standard curve.
   Sv is the sample volume (µl) 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:

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

DATA SHEET

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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