BioVision

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For research use only

Lactate Colorimetric Assay Kit II

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

I. Introduction:

Lactate ($CH_3CH(OH)COO^-$) 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 serum or plasma, cells, culture and fermentation media at a level of 0.05 mM- 20 mM. There is no need for pretreatment or purification of 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 µl	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:

1. **Standard Curve Preparations:** Dilute the Lactate Standard (MW 90.08) to 1 mM 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.
2. **Sample Preparation:** Prepare test samples at 50 µl/well with Lactate Assay Buffer in a 96-well plate. For serum samples, 0.5-10 µl serum can be directly tested (regular serum contains ~0.6 nmol/µl 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 10kDa MW spin filter (BioVision, Cat.# 1997-25) to remove all proteins.
3. **Reaction Mix Preparation:** Mix sufficient 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 Lactate Substrate Mix
   2 µl Lactate 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 min at room temperature.
6. Measure OD 450nm in a microplate reader. The color is stable for at least 4 hrs.
7. **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 (nmol/µl or mM)

   Where: La is the lactic acid amount (nmol) of your sample from standard curve.
   Sv is the sample volume (µl) added into the well.
   Lactic acid molecular weight: 90.08.

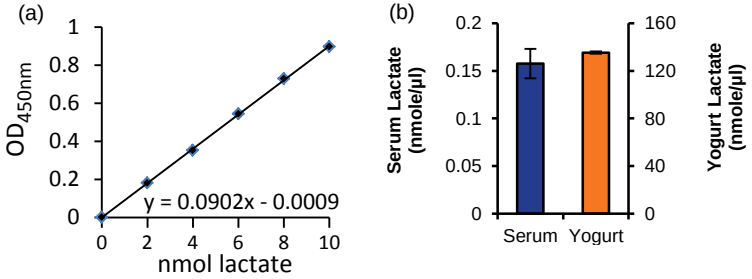

Figure: Lactate Standard Curve (a). (b) Lactate estimation in pooled human Serum and yogurt. Undiluted serum was used in the assay as described in the kit protocol. Deproteinizing Sample Preparation Kit (Cat. # K808) was used to remove protein from the yogurt and the supernatant was diluted 1:200 - 1:500. Assay was performed following the kit protocol.

RELATED PRODUCTS:

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

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BioVision Incorporated
155 S. Milpitas Boulevard, Milpitas, CA 95035 USA
Tel: 408-493-1800 | Fax: 408-493-1801
www.biovision.com | tech@biovision.com

GENERAL TROUBLESHOOTING GUIDE:

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.		