Product Information

Lactate Colorimetric Assay Kit II

I. Kit Contents:

Components	K2131-100 100 assays	Cap Color	Part Number
Lactate Assay Buffer	25 ml	WM	K2131-C-1
Lactate Enzyme Mix	lyophilized	Green	K2131-C-2
Lactate Substrate Mix	lyophilized	Red	K2131-C-3
L(+)-Lactate Standard (100 mM	100 µl	Yellow	K2131-C-4

II. Introduction:

Lactate is chiral: L(+)-Lactate and D(-)-Lactate. L(+)-Lactate exists in blood and is constantly produced from pyruvate by lactate dehydrogenase (LDH) in human intermediary metabolism. D(-)-Lactate is present only at about 1 - 5% of the concentration of L(+)-Lactate. Abnormal high concentration of lactate is related to diseases such as lactate acidosis and diabetes.

The Lactate Colorimetric Assay Kit II provides a sensitive, simple and convenient way for detection of lactate in various biological samples (plasma, serum, culture and fermentation media, cells, etc.) based on colorimetric method. In the assay, lactate is oxidized by lactate dehydrogenase to product a product which interacts with a probe to yield a color (λmax = 450 nm). The assay can be performed without pretreatment or purification of samples. The kit can detect 0.02 mM - 10 mM lactate in various samples.

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℃. 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℃.

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℃ for storage, or filtered through a 10 kDa MW spin filter 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:

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

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.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not	• Use of partially thawed components	• Thaw and resuspend all components before preparing the

follow a linear pattern for Standard curve	• Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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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