Product Information

D-Lactate Colorimetric Assay Kit

I. Kit Contents:

Components	K2213-100 100 assays	Cap Color	Part Number
D-Lactate Assay Buffer	25 ml	WM	K2213-C-1
D-Lactate Enzyme Mix	lyophilized	Green	K2213-C-2
D-Lactate Substrate Mix	lyophilized	Red	K2213-C-3
D-Lactate Standard (100 mM)	100 µl	Yellow	K2213-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 mainly produced through the glyoxalase pathway in mammals. D(-)-Lactate is present only at about 1-5% of the concentration of L(+)-Lactate and is extremely low with normal serum concentrations in the nM to µM range. The elevated D-lactate levels which can rise to mM levels are caused by short bowel syndrome or bacterial infection in humans. Abnormal high concentration of D-lactate is related to diseases such as sepsis, ischemia or trauma. Because of slow metabolism and excretion, high D-lactate can cause acidosis and encephalopathy.

The D-Lactate Colorimetric Assay Kit provides a simple, fast and convenient way for accurate detection of D-lactate levels in various biological samples (serum, plasma, cells, culture and fermentation media) based on colorimetric method. In the assay, D-lactate is specifically oxidized by D-lactate dehydrogenase and produces proportional color (λmax = 450 nm). The kit can detect the useful D-lactate concentration range 0.01 mM - 10 mM in various samples.

III. Reagent Preparation and Storage Conditions:

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

Substrate Mix: Reconstitute with 0.22 ml of D-Lactate Assay Buffer and mix thoroughly. The solution is stable for two months at 4℃.

IV. Lactate Assay Protocol:

1. Standard Curve Preparations: Dilute the 100mM D-Lactate Standard to 1 mM by adding 10 µl of the Standard to 990 µl of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the D-Lactate Standard.

2. Sample Preparation: Prepare 1 - 50 µl test samples in a 96-well plate. Adjust the volume to 50 µl /well with Assay Buffer. We suggest using several doses of your sample to ensure the readings are within the standard curve range.

Note:

(1) Tissue (20 mg) or cells (2 x 10^6) can be homogenized in 100 µl the Assay Buffer. Centrifuge at 10,000 x g for 10 min to remove insoluble materials. The soluble fraction may be assayed directly.

(2) Endogenous enzyme activity may cause loss of D-lactate. Samples containing enzyme activity (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 reagents for the number of assays performed. For each well, prepare a total 50 µl Reaction Mix containing the following components. Mix well before use:

D-Lactate Assay Buffer 46 µl

D-Lactate Substrate Mix 2 µl

D-Lactate Enzyme Mix 2 µl

Note: NADH or NADPH from cell or tissue extracts generates background for the lactate assay. To subtract the NADH or NADPH background, same amount of sample can be tested in the absence of Enzyme Mix, which detect NAD(P)H, not D-Lactate. Then the background readings can be subtracted from the D-lactate reading.

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

5. Incubate the reaction for 30 min at room temperature.

6. Measure $OD_{450\ nm}$ in a microplate reader. The color is stable for at least 4 hours.

7. Calculation: Correct background by subtracting the value derived from the 0 D-lactate control from all standard and sample readings (Note: Background can be significant and must be subtracted from all readings). Plot a standard curve of nmol/well vs. $OD_{450\ nm}$. Apply the sample readings to the standard curve. Calculate the D-Lactate concentrations of the test samples:

C = La/Sv (nmol/µl, µmol/ml or mM)

Where: La is the D-lactate amount (nmol) of your sample from the standard curve.

Sv is the sample volume (µl) added into the well.

D-Lactic acid molecular weight: 90.08.

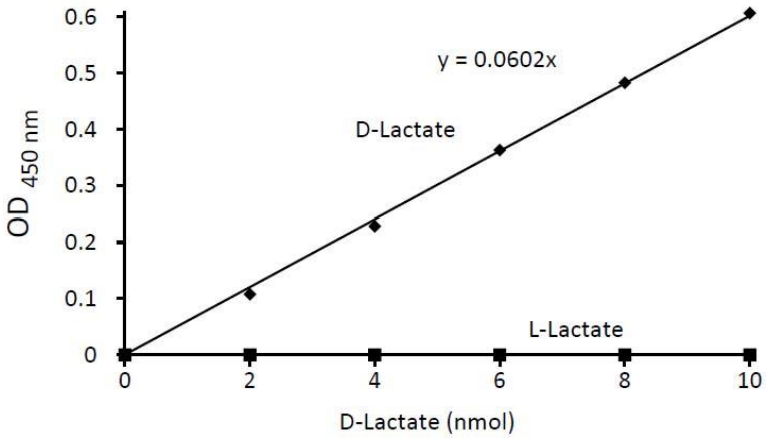

Fig. 1. D-Lactate Standard Curve. The assay is performed according to the kit instruction. The assay specifically detects D-Lactate, not L-Lactate, in the presence of up to 1000X of L-Lactate.

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	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet

	• 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	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 follow a linear pattern for Standard curve	• Use of partially thawed components • 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	• Thaw and resuspend all components before preparing the 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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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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