BioVision

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For research use only

D-Lactate Colorimetric Assay Kit

(Catalog #K667-100; 100 assays; Store kit at –20˚C)

I. Introduction:

D-Lactate production in mammals, mainly due to the glyoxalase pathway, is extremely low, with normal serum concentrations in the nano to micromolar range. Typically, elevated D-lactate levels which can rise to millimolar levels are due to bacterial infection or short bowel syndrome in humans. Abnormally high concentrations of D-lactate are considered indicative of sepsis, ischemia or trauma. Due to slow metabolism and excretion, high D-lactate can cause acidosis and encephalopathy. BioVision's D-Lactate Assay Kit provides a fast, easy way to accurately measure D-lactate in a variety of biological samples. In the D-Lactate Assay Kit, D-lactate is specifically oxidized by D-lactate dehydrogenase and generates proportional color (λ_{max} = 450 nm). The kit detects D-Lactate in samples such as serum, plasma, cells, culture and fermentation media. The useful concentration range in samples is 0.01 mM – 10 mM D-lactate.

II. Kit Contents:

Components	K667-100	Cap Color	Part Number
D-Lactate Assay Buffer	25 ml	WM	K667-100-1
D-Lactate Enzyme Mix	lyophilized	Green	K667-100-2
D-Lactate Substrate Mix	lyophilized	Red	K667-100-3
D-Lactate Standard (100 mM)	100 µl	Yellow	K667-100-4

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°C. 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°C.

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°C for storage, or filtered through a 10 kDa mw spin filter (BioVision, Cat.# 1997-25) 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:

   46 µl D-Lactate Assay Buffer
   2 µl D-Lactate Substrate Mix
   2 µl D-Lactate Enzyme Mix **

** **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

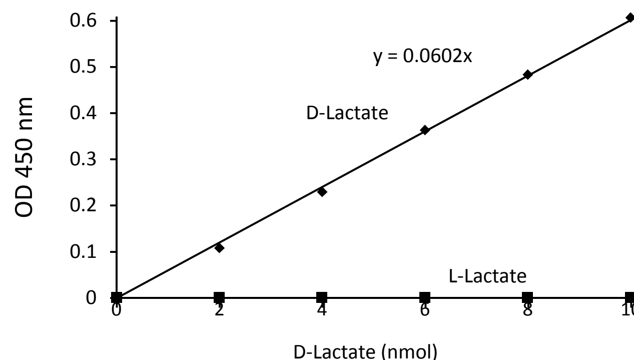

Fig. 1. D-Lactate Standard Curve. The assay is performed according to the kit instruction. The assay specifically detects D-Lactate, not L-Lactate, in the presence of up to 1000X of L-Lactate.

V. Related Products:

Cholesterol Assay Kit	Glutathione Assay Kit
Glucose Assay Kit	Cytotoxicity Assay
Pyruvate Assay Kit	Creatinine Assay Kit
Apoptosis Assay Products	Cell Proliferation Assays
Starch Assay Kit	NADH/NADPH Assay Kits
L-Lactate II Colorimetric Assay	L-Lactate Fluorometric/Colorimetric Assay Kits
Ascorbic Acid Assay Kit	Glycogen Assay kit
ADP & ATP Assay Kits	Creatinine & Creatine Assay Kits
Ethanol Assay Kit	Uric Acid Assay Kit
Nitric Oxide Assay Kits	HDL & LDL Assay Kit
Sarcosine Assay Kit	Antioxidant Assay Kit
Triglyceride Assay Kit	Glycerol Assay Kit
Ammonia Assay Kit	Urea Assay Kit

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay 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 (clear bottoms) ; 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 datasheet) • 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 datasheet & 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.		