

# 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 ( $\lambda_{max} = 450 \text{ nm}$ ). 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 $\mu\text{l}$	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:**

- Standard Curve Preparations:** Dilute the 100mM D-Lactate Standard to 1 mM by adding 10  $\mu\text{l}$  of the Standard to 990  $\mu\text{l}$  of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  into a series of wells. Adjust volume to 50  $\mu\text{l}$ /well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the D-Lactate Standard.
- Sample Preparation:** Prepare 1-50  $\mu\text{l}$  test samples in a 96-well plate. Adjust the volume to 50  $\mu\text{l}$  /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 ( $2 \times 10^6$ ) can be homogenized in 100  $\mu\text{l}$  the Assay Buffer. Centrifuge at 10,000g 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 10Kd mw spin filter (BioVision, Cat.# 1997-25) to remove all proteins.

- Reaction Mix Preparation:** Mix sufficient reagents for the number of assays performed. For each well, prepare a total 50  $\mu\text{l}$  Reaction Mix containing the following components. Mix well before use:

- 46  $\mu\text{l}$  D-Lactate Assay Buffer
- 2  $\mu\text{l}$  D-Lactate Substrate Mix
- 2  $\mu\text{l}$  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.

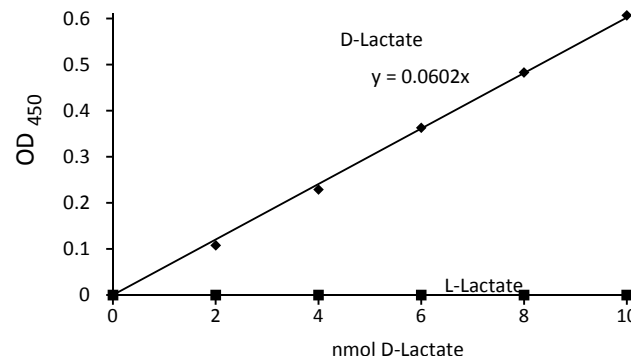
- Add 50  $\mu\text{l}$  of the Reaction Mix to each well containing the D-Lactate Standard or test samples, mix well.
- Incubate the reaction for 30 minutes at room temperature.
- Measure O.D. 450 nm in a microplate reader. The color is stable for at least 4 hours.
- Calculation:** Correct background 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<sub>450nm</sub>. Apply the sample readings to the standard curve. Calculate the D-Lactate concentrations of the test samples:

$$C = La/Sv \text{ (nmol/}\mu\text{l, }\mu\text{mol/ml or mM)}$$

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

**Sv** is the sample volume ( $\mu\text{l}$ ) 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 in the presence of up to 1000X L-Lactate.

**V. Related Products:**

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|---------------------------------|--|
| 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                                 |