FOR RESEARCH USE ONLY!

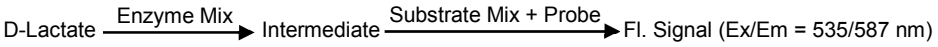
PicoProbe™ D-Lactate Fluorometric Assay Kit

rev. 4/13

(Catalog # K668-100; 100 assays; Store at -20°C)

I. Introduction:

D-Lactate is the result of anaerobic glycolysis by microorganisms in the gastrointestinal system, and the product of detoxification of methylglyoxal by the glyoxalase system. The presence of abnormal levels of D-Lactate has been linked to a series of pathological conditions, such as diabetes, and appendicitis. BioVision's PicoProbe™ D-Lactate Assay kit offers simplicity, sensitivity, and can be adapted to high-throughput research. The assay enzymatically oxidizes D-Lactate generating a fluorescent signal (Ex/Em = 535/587 nm). The signal is directly proportional to the amount of D-Lactate. The assay kit can detect D-Lactate as low as 0.1 µM in a variety of samples.



II. Application:

- Measurement of D-Lactate in various tissues/cells/biological fluids
- Analysis of D-lactate in pathological conditions
- Mechanistic study of the glyoxalase system

III. Sample Type:

- Serum, plasma, urine & other body fluids
- Animal tissues: liver, muscle, heart, etc.
- Cell culture: adherent or suspension cells
- Fermentation media
- Food

IV. Kit Contents:

Components	K668-100	Cap Code	Part Number
D-Lactate Assay Buffer	25 ml	WM	K668-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K668-100-2
D-Lactate Enzyme Mix (Lyophilized)	1 vial	Green	K668-100-3
D-Lactate Substrate Mix (Lyophilized)	1 vial	Red	K668-100-4
D-Lactate Standard (100 mM)	0.1 ml	Yellow	K668-100-5

V. User Supplied Reagents and Equipment:

- White 96-well plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm D-Lactate Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **D-Lactate Enzyme Mix**: Reconstitute with 220 µl D-Lactate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.
- **D-Lactate Substrate Mix**: Reconstitute with 220 µl D-Lactate Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.

VIII. D-Lactate Assay Protocol:

1. **Sample Preparation:** Prepare 2-50 µl test samples in a 96-well plate (for high target concentrations, dilute the samples). Bring the volume to 50 µl/well with D-Lactate Assay Buffer. We suggest using different volumes of your sample to ensure the readings are within the Standard Curve range.

   Notes:

   (a) Tissue (20 mg) or cells (2 x 10^6) can be homogenized in 100 µl D-Lactate Assay Buffer. Centrifuge at 10,000x g for 10 min. to remove insoluble material. Soluble fractions may be assayed directly.

   (b) Body fluids, such as urine, should be filtered using a 10 kDa MW spin filter (BioVision, Cat # 1997-25). Dilute samples with pure water. An appropriate dilution factor is usually between 1:10 and 1:100. Diluted samples can be assayed directly.

   (c) Food samples. A) Beer: Remove CO_2 by vacuum-filtering samples. Dilute samples with pure water (appropriate dilution factor is approximately 1:10). Diluted samples can be assayed directly. B) Yogurt: vortex 1 gram of yogurt in 10 ml of water until homogeneous. Dilute with pure water (dilution factor ~ 1:100 to 1:500). Diluted samples can be assayed directly.

   (d) 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 or filtered through a 10 kDa MW spin filter (BioVision, Cat.# 1997-25).

2. **Standard Curve Preparation:** Dilute D-Lactate Standard to 1 mM (1000 pmol/µl) by adding 10 µl of 100 mM D-Lactate Standard to 990 µl D-Lactate Assay Buffer, mix well. Dilute further to 0.02 mM (20 pmol/µl) by adding 20 µl of 1 mM D-Lactate Standard to 980 µl of D-

Lactate Assay Buffer. Mix well. Add 0, 2, 4, 6, 8 & 10 µl of 0.02 mM D-Lactate Standard into a series of wells in a 96 well plate to generate 0, 40, 80, 120, 160 and 200 pmol/well of D-Lactate Standard. Adjust volume to 50 µl/well with D-Lactate Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
D-Lactate Assay Buffer	45 µl	47 µl
PicoProbe™	1 µl	1 µl
D-Lactate Enzyme Mix	2 µl	----
D-Lactate Substrate Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing Standard & test samples. Mix well.

***Note:** For samples having high NADH levels, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement:** Incubate the reaction for 30 minutes at 37°C, protected from light. Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader with kinetics mode.

5. **Calculation:** Subtract 0 D-Lactate Standard reading from all readings. Plot the D-Lactate Standard curve. If sample background control reading is significantly high, subtract the background control reading from sample reading. Apply the corrected sample reading to the D-Lactate Standard curve to get B pmol of D-Lactate in the sample wells.

Sample D-Lactate concentration = B/V x Dilution Factor = pmol/µl = nmol/ml or µM

Where: **B** = amount of D-Lactate in sample well from Standard curve (pmol)

V = sample volume added in the reaction well (µl)

D-Lactate in samples can also be expressed in nmol/mg of sample.

D-Lactate molecular weight: 112.1 g/mol.

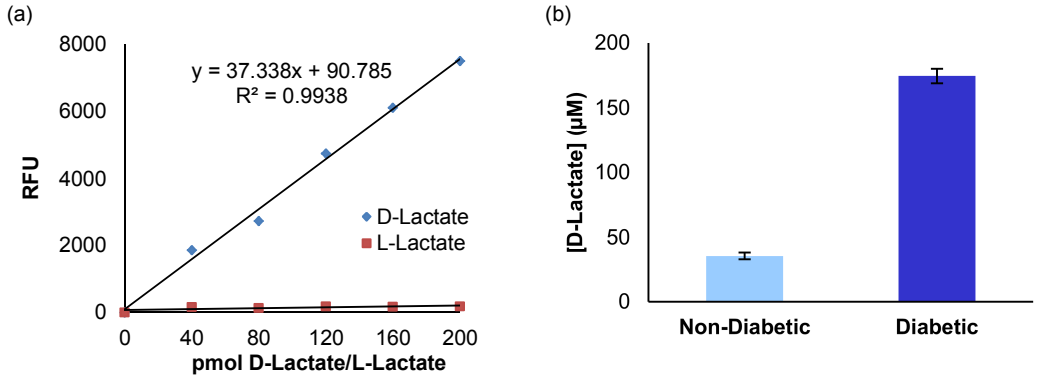


Figure: (a) D-Lactate Standard Curve. The assay specifically detects D-Lactate, not L-Lactate. (b) Measurement of D-Lactate in urine: Diluted samples (1:10, non-diabetic; 1:100 diabetic) were spiked with known amounts of D-Lactate (0-200 pmol) and assayed as specified.

IX. RELATED PRODUCTS:

Lactate Colorimetric Assay Kit II
PicoProbe™ Lactate Fluorometric Assay Kit
Lactate Dehydrogenase (LDH) Activity Assay Kit
Human Recombinant LDHA
LDH Antibody
Glutathione Assay Kit
Cytotoxicity Assay Kit
Creatinine Assay Kit
NADH/NADPH Assay Kits
Lactate Colorimetric/Fluorometric Assay Kit
D-Lactate Colorimetric Assay Kit
LDH-Cytotoxicity Colorimetric Assay Kit II
Human Recombinant LDHB
Cholesterol Assay Kit
Glucose Assay Kit II
Pyruvate Assay Kit
Cell Proliferation Assays

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