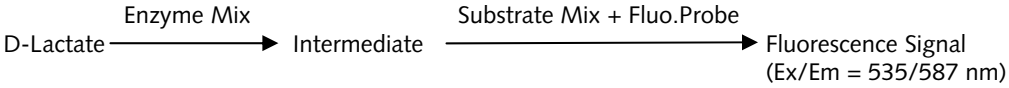
D-Lactate Assay Kit II
(fluorometric)

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K668
Description	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. PromoKine's 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.  **Application:** • Measurement of D-Lactate in various tissues/cells/biological fluids • Analysis of D-lactate in pathological conditions • Mechanistic study of the glyoxalase system **Sample Type:** • Serum, plasma, urine & other body fluids • Animal tissues: liver, muscle, heart, etc. • Cell culture: adherent or suspension cells • Fermentation media • Food
Quantity	100 assays

Kit Components

Component	Quantity	Cap Code
D-Lactate Assay Buffer	25 ml	WM
Lactate Fluorometric Probe (in DMSO)	0.4 ml	Blue
D-Lactate Enzyme Mix (lyophilized)	1 vial	Green
D-Lactate Substrate Mix (Lyophilized)	1 vial	Red
D-Lactate Standard (100 mM)	0.1 ml	Yellow

User Supplied Reagents and Equipment:

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

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.

Reagent Preparation and Storage Conditions:

- **Lactate Fluorometric Probe:** 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.

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 minutes 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 (Cat # PK-CA577-1997). 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 (Cat.# PK-CA577-1997).

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 and 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 and 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/>l = nmol/ml or >M

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.

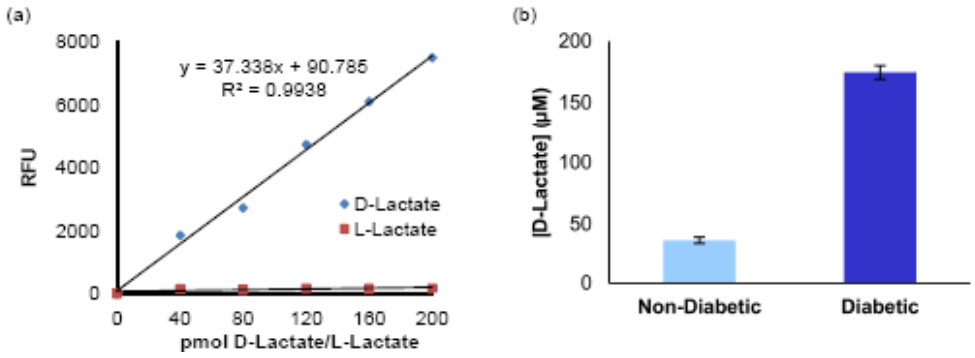


Figure: (a) D-Lactate Standard Curve. The assay specifically detects D-Lactate, not L-Lactate. (b) Measurement of Dlactate in urine: Diluted samples (1:10, non-diabetic; 1:100 diabetic) were spiked with known amounts of D-Lactate (0-200 pmol) and assayed as specified.

Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

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12/2014