EZ-Lactate Assay Kit

Metabolism Assay Kit

(Colorimetric/Fluorometric)

Cat. No. DG-LAC100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Lactate is a metabolite produced by lactate dehydrogenase in the absence or low oxygen environment, and plays an important role in biological processes as an indicator of the state of metabolism and circulation by fluctuating in certain diseases. Lactate exists as two optical isomers, L(+) and D(-), but in the human body, L-lactate, the L form, is mainly produced and exists, and only about 1 to 5% exists as the D form.

The EZ-Lactate assay kit can directly detect the concentration of L-Lactate in a sample by using an enzymatic reaction that specifically reacts with L-Lactate. The probe used can use both absorption (O.D. 570 nm) and fluorescence (Ex/Em = 535/590 nm) methods simultaneously, allowing the user to select a method that suits their equipment or detection sensitivity.

▪ Kit Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage (reconstituted)
Lactate Assay buffer	25 ㎖	-	-20 ℃, 2개월
Lactate Enzyme mix (Lyophilized)	1 vial	Red	-20 ℃, 2개월
Lactate Probe	200 ㎕	Yellow	-20 ℃, 2개월
L(+)-Lactate Standard (100mM)	100 ㎕	Green	-20 ℃, 2개월

* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Sample type

- Serum, Plasma, Blood
- Animal tissue, Cell line

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
Lactate Enzyme mix	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20°C.
Lactate Probe	Use after sufficiently dissolving at room temperature.	The remaining solution can be stored at -20°C, but use within 2 months.
L(+)-Lactate Standard	Use after sufficiently dissolving at room temperature.	The remaining solution can be stored at -20°C, but use within 2 months.

* Assay buffer is used after sufficiently warming up to room temperature before experiment.
* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

▪ General Protocol

1. Standard preparation

1) Colorimetric method

Prepare a 1 mM Lactate standard solution by mixing 10 ㎕ of 100 mM Lactate standard and 990 ㎕ of Assay buffer. Dispense 0, 2, 4, 6, 8, and 10 ㎕ of the standard solution into a 96-well plate and adjust the final volume to 50 ㎕ with Assay buffer. This will result in standards of 0, 2, 4, 6, 8, and 10 nmol/well in each well.

Standard No.	Volume of 1 mM L(+)-Lactate standard	Assay buffer	Final standard volume in well	Final standard amount in well (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	2
3	4 ㎕	46 ㎕	50 ㎕	4
4	6 ㎕	44 ㎕	50 ㎕	6
5	8 ㎕	42 ㎕	50 ㎕	8
6	10 ㎕	40 ㎕	50 ㎕	10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.
* Standard recommends measurement during each experiment.

2) Fluorometric method

Prepare a 1 mM Lactate standard solution by mixing 10 ㎕ of 100 mM Lactate standard and 990 ㎕ of Assay buffer. Prepare a 0.01 mM Lactate standard solution by mixing 10 ㎕ of diluted 1 mM Lactate standard and 990 ㎕ of Assay buffer. Dispense 0, 2, 4, 6, 8, and 10 ㎕ of the standard solution into a 96-well plate and adjust the final volume to 50 ㎕ with Assay buffer. Each well will have standards of 0, 0.02, 0.04, 0.06, 0.08, and 0.10 nmol/well.

Standard No.	Volume of 0.01 mM L(+)-Lactate standard	Assay buffer	Final standard volume in well	Final standard amount in well (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	0.02
3	4 ㎕	46 ㎕	50 ㎕	0.04
4	6 ㎕	44 ㎕	50 ㎕	0.06
5	8 ㎕	42 ㎕	50 ㎕	0.08
6	10 ㎕	40 ㎕	50 ㎕	0.10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2. Sample preparation

Add 2-50 ㎕ of the prepared sample to a 96-well plate, and adjust the final volume to 50 ㎕ with Assay buffer. (n≥2)

1) Cell or Tissue

① Prepare 2~20 x 10^6 cells or approximately 10 mg tissue sample.

② Wash the sample using PBS.

③ Add Assay buffer solution to the sample and homogenize on ice.

④ Centrifuge at 10,000xg at 4℃ for 10 minutes and use the supernatant for analysis.

2) Serum, plasma, blood

Dilute and use with Assay buffer without preprocessing.

3) For unknown samples or samples being measured for the first time, ensure that the measured values are within the standard curve. We recommend using it after preliminary testing.

4) For samples with high background, prepare the same amount of sample used for measurement as a sample background control.

3. Reaction mixture preparation

* Colorimetric method / Fluorometric method are both proceed in the same way.

This is the volume for 1 assay. This is the volume based on one well assay. Calculate the assay volume to be used in the experiment and prepare the reaction mix considering the loss volume.

Components	Reaction mixture	Background Reaction mixture
Lactate Assay buffer	46 ㎕	48 ㎕
Lactate Enzyme mix	2 ㎕	-
Lactate Probe	2 ㎕	2 ㎕
Total	50 ㎕	50 ㎕

4. Add 50㎕ of the reaction mixture to each well containing the L-Lactate standard and the experimental material using a multi pipette and mix well.

* Colorimetric method / Fluorometric method are both proceed in the same way

* If you have prepared a background control, add the background reaction mixture to the background control well.

5. After incubation the plate at room temperature blocked from light for 30 minutes, shake gently and measure using a microplate reader.

1) Colorimetric : 570 nm
2) Fluorometric : (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

1. Subtract the O.D. value of the blank from all measurements.
   * Blank = OD_{570nm} from Lactate standard #1 (0 nmol Lactate)
2. Determine the standard curve using Lactate standard measurements.
3. Calculate the concentration for each sample using the linear equation represented by the standard curve.
4. The amount of lactate is calculated by substituting the sample measurement values into the standard curve.

* If background control is set, the amount of lactate is calculated by subtracting the background control measurement value from the sample measurement value.

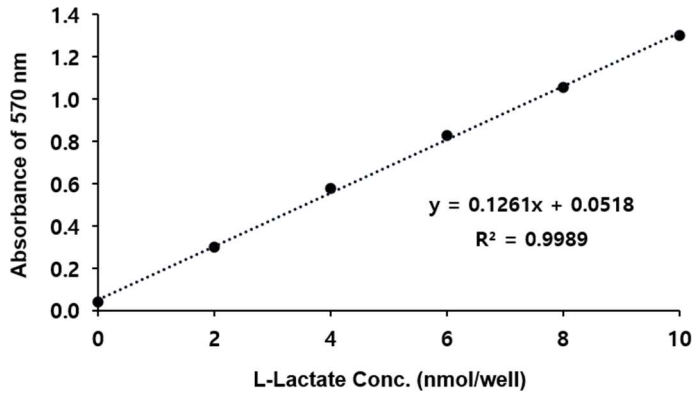
5. Based on the amount of lactate calculated in 4, the concentration of lactate in the sample is calculated using the following equation.

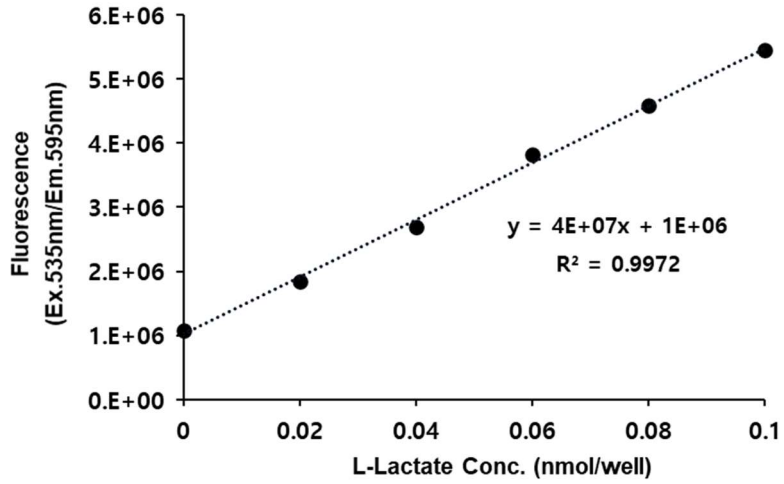
Lactate concentration in sample (C) = B/V x D (nmol/㎕ or mM)

B : Amount of lactate in the measurement well obtained from the standard curve (nmol)

V : Amount of sample in a well (㎕)

D : Sample dilution ratio (ex. If diluted 2x, calculate as x2, not x1/2.)





Lactate standard curve. Assay was performed following the kit protocol.

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▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay