Lactate Assay Kit-WST

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/I256.pdf

General Information

Lactate is a metabolite of glycolysis that is one of the main metabolic pathways in cells, and is known to be a biomarker for muscular fatigue and hyperlactacidemia. It also serves as a marker for monitoring the changes of intracellular metabolic pathways. In addition, recent metabolomic study suggests that lactate contributes as a major carbon source in the TCA cycle of tissues and cancer cells[1].

Lactate Assay Kit-WST enables quantitation of lactate produced by glycolysis. This kit has been optimized to quantitate lactate in cell culture supernatant by measuring the absorption derived from a colorimetric reaction of WST. This kit is formated for 96-well microplate assays with a detection sensitivity limit of 0.02 mmol/l lactate.

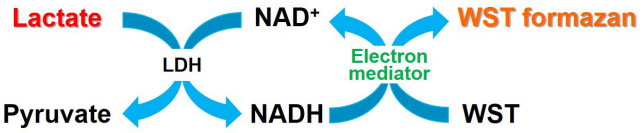

Fig. 1 Principle of Lactate Assay Kit-WST

Kit Contents

	50 tests	200 tests
Dye Mixture	× 1	× 1
Lactate Standard (10 mmol/l)	150 µl × 1	600 µl × 1
Enzyme Solution	12 µl × 1	48 µl × 1
Assay Buffer	5.5 ml × 1	11 ml × 2
Reconstitution Buffer	550 µl × 1	2.2 ml × 1

Storage Condition

Store at 0-5°C

Required Equipment

- Microplate reader (450 nm filter)
- Incubator (37°C)
- 20 µl, 200 µl, 1000 µl micropipettes
- 96-well microplate
- 20-200 µl multichannel pipette

Precautions

- Equilibrate the kit to room temperature prior to use.
- Pipete the Enzyme Solution before use to obtain the homogenous mixture since an enzyme is suspended in a liquid.
- Triplicate measurement per sample is recommended to obtain accurate data.
- Since the enzymatic reaction starts immediately after the addition of Working solution to a well, use a multichannel pipette to minimize the experimental error from time lag in pipetting.
- Please prepare samples with different dilution rate and determine the suitable dilution rate to be ranging from 0 to 1 mmol/l.
- A glass bottle and an aluminum cap are used as a package of Dye Mixture. Use protective gloves with cautious in handling.
- This kit is designed for measuring cell culture supernatant samples. For measuring a concentration of intracellular lactate, use 0.1% Triton solution for preparation of cell lysate and Lactate standard solution.

Preparation of Solutions

Preparation of Dye Mixture stock solution

Add all Reconstitution Buffer to a Dye Mixture vial. Close the cap and dissolve the contents completely.

※ Transfer the Dye Mixture stock solution to the vial of the Reconstitution Buffer and store it at 0-5°C with protection from light. Dye Mixture stock solution is stable for 4 months under these conditions.

Preparation of Working solution

(1) Add Dye Mixture stock solution to a conical tube and dilute it with Assay Buffer.

(2) Add Enzyme Solution to the solution prepared in step (1).

※ Refer to Table 1.

※ Working solution is light sensitive. Prepare the solution just before use and protect it from light by covering with aluminum foil. Please use up Working solution within that day.

	for 24 well	for 48 well	for 96 well
Dye Mixture stock solution	250 µl	500 µl	1 ml
Assay Buffer	2.25 ml	4.5 ml	9 ml
Enzyme Solution	5 µl	10 µl	20 µl

Table. 1 Examples of preparation of Working solution

General Protocol

1. Sample preparation

Prepare cell culture supernatant samples (Sample).

※ Please prepare samples with different dilution rate and determine the suitable dilution rate to be ranging from 0-1 mmol/l. Use double-deionized H_2O (ddH_2O) for diluting.

※ In case a medium contains serum, read the blank absorbance (serum containing medium) as background control and subtract its value from absorbance of each sample.

※ Required sample amount is 20 µl for each well.

2. Preparation of Lactate standard solution

Mix 50 µl of 10 mmol/l Lactate Standard and 450 µl of ddH_2O in a microtube to prepare a 1 mmol/l Lactate standard solution. Prepare the following Lactate standard solution by serial dilution with ddH_2O: 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0157 and 0 mmol/l (Fig. 2).

※ For measuring a concentration of intracellular lactate, prepare Lactate standard solution with 0.1% Triton solution instead of ddH_2O.

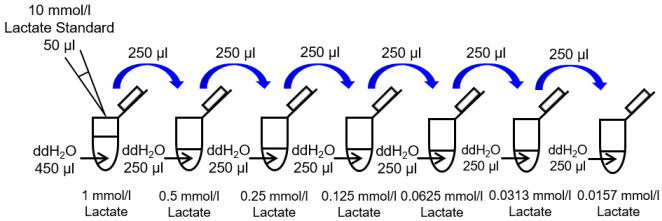

Fig. 2 Preparation of Lactate standard solution

3. Measurement

(1) Add 20 µl of Lactate standard solution and sample solutions to each well (Fig. 3).

※ In order to obtain accurate data, we recommend triplicate measurement per sample.

(2) Add 80 µl of Working solution to each well.

※ Since the enzymatic reaction starts immediately after the addition of Working solution to the well, use a multichannel pipette to minimize the experimental error from time lag in pipetting.

(3) Incubate the microplate at 37°C for 30 minutes.

※ Use a seal for the microplate to prevent evaporation of the solution during the incubation.

(4) Measure the absorbance at 450 nm by using a microplate reader.

(5) Determine the concentration of lactate in the sample using a calibration curve.

※ If the original samples have been diluted for this assay, multiply the determined value and dilution rate.

	1	2	3	4	5	6
A	0 mmol/l Lactate			Sample 1		
B	0.0157 mmol/l Lactate			Sample 2		
C	0.0313 mmol/l Lactate			Sample 3		
D	0.0625 mmol/l Lactate			Sample 4		
E	0.125 mmol/l Lactate			Sample 5		
F	0.25 mmol/l Lactate			Sample 6		
G	0.5 mmol/l Lactate			Sample 7		
H	1 mmol/l Lactate			Sample 8		

Fig. 3 An example of plate arrangement (n=3)

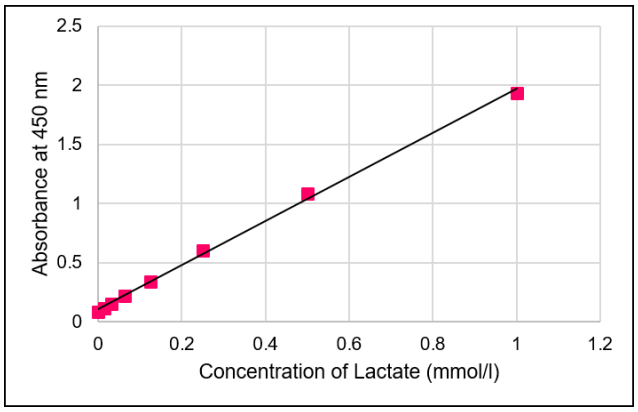

Fig. 4 Typical calibration curve of lactate

Experimental Example

Glycolysis inhibition by 2-deoxy-D-glucose

(1) HeLa cells (1×10^4 cells/well, MEM containing 10% fetal bovine serum and 1% penicillin-streptomycin) were seeded in a 96-well microplate and cultured overnight in a 5% CO_2 incubator.

(2) After the removal of supernatant, 100 µl of medium containing 2-deoxy-D-glucose was added.

(3) The cells were cultured overnight in the 5% CO_2 incubator.

(4) After the incubation, 20 µl of the cell culture supernatant was transferred to a 1.5-ml microtube and diluted 8 times with ddH_2O to prepare the sample solution, and then 20 µl of the sample solution was added to each well.

(5) Working solution (80 µl) was added to each well.

(6) The 96-well microplate was incubated at 37°C for 30 minutes.

(7) The absorbance at 450 nm was measured by using a microplate reader, and the concentration of lactate in the sample was determined using a calibration curve.

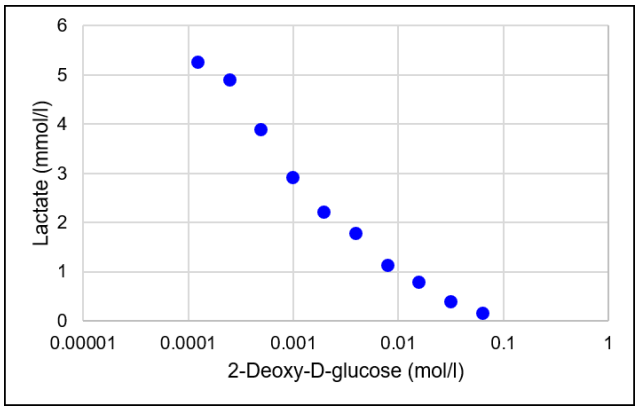

Fig. 5 Glycolysis inhibition by 2-deoxy-D-glucose

Lactate concentration decreased with increasing concentrations of 2-deoxy-D-glucose (one of the glycolysis inhibitors).

Reference

1) S.Hui, *et al*., *Nature*, **2017**, *551*, 115.

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