Lactate Dehydrogenase (LDH) Activity Assay Kit

Catalog Number EEA013 (96 tests)

Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to measure lactate dehydrogenase (LDH) activity in tissues, serum (plasma) and culture cell samples. Lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells (animals, plants, and prokaryotes) which catalyzes the cyclic conversion of lactate to pyruvic acid, as it converts NAD^+ to NADH by transferring hydrides between molecules. LDH is expressed extensively in body tissues, such as blood cells and heart muscle. LDH is released during tissue damage, and can be considered a marker of common injuries and disease such as heart failure. Lactate dehydrogenase is composed of four subunits (tetramer), where the two most common subunits are the LDH-M and LDH-H protein, encoded by the LDHA and LDHB genes, respectively. These two subunits can form five possible tetramers (isoenzymes): 4H, 4M, and the three mixed tetramers (3H1M, 2H2M, 1H3M). These five isoforms are enzymatically similar but show different tissue distribution: The major isoenzymes of skeletal muscle and liver, M4, has four muscle (M) subunits, while H4 is the main isoenzymes for heart muscle in most species, containing four heart (H) subunits.

Using coenzyme I as a hydrogen carrier, LDH catalyze lactic acid to produce pyruvate. Pyruvate reacted with 2, 4-dinitrophenylhydrazine to form pyruvate dinitrophenylhydrazone, which is red-brown in alkaline solution, and the color intensity is proportional to pyruvate concentration. The activity of LDH can be calculated by measuring OD value.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Substrate Buffer	5 mL
Coenzyme I	6 mg
Chromogenic Agent	5 mL
Alkali Reagent	5 mL
2 μmol/mL Pyruvic Acid Standard	1 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- Avoid using hemolytic serum samples, as the LDH activity in red blood cells is about 100 times higher than that in serum.
- SDS, Tween 20, NP-40, Triton X-100 and other detergents should not be included in the sample.
- Oxalate anticoagulants should not be used because oxalate will inhibit the activity of LDH.

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month

Cells:

- Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1 times.
- Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment.
- Add homogenization medium at a ratio of cell number (10^6): PBS (0.01 M, pH 7.4) (μL) =1: 300-500.
- Homogenize cells by an ultrasonic cell disruptor(200 W, 2 s/time, interval for 3 s, the total time is 5 min) or homogenizer instrument (60 Hz 90s) on ice

- Centrifuge at 10,000 g for 10 min at 4℃, then take the supernatant and preserve it on ice for detection.
  Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).
  If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for 1 month.

Prepare samples

It is recommended to take 2-to-3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (6-1000 U/L).

Note: Use all samples within 2 hours of dilution

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	10
Human plasma	15-30
Porcine serum	10-20
10% Mouse kidney tissue homogenization	500-800
10% Mouse lung tissue homogenization	500
10% Mouse liver tissue homogenization	500-800
HepG2 cells homogenization	100-300

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Preparation of coenzyme I application solution

Dissolve vial of powder with 1.33 mL distilled water, where it can be stored at -20 ℃ for 2 weeks. It is recommended to aliquot the prepared solution and store at -20 ℃

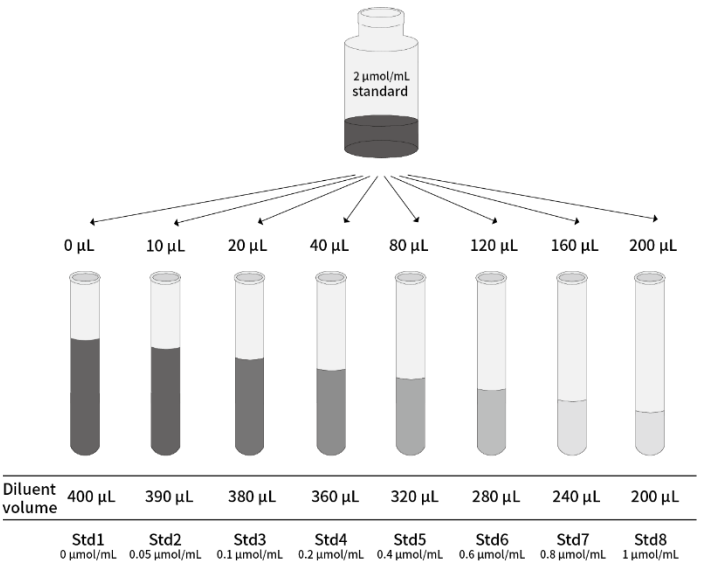
Preparation of alkali reagent application solution

Prepare fresh solution before use by mixing 1 part of alkali reagent with 9 parts of distilled water.

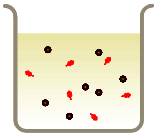
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 2 μmol/mL pyruvic acid standard solution with distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 μmol/mL.



Assay Procedure



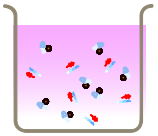
1. Add standard, sample and control

a. Standard well: add 5 μL of double distilled water and 20 μL of pyruvic acid standard solution with different concentrations.

b. Sample well: add 20 μL of sample.

c. Control well: add 5 μL of double distilled water and 20 μL of sample.

d. Add 25 μL of substrate buffer to each well.



2. Add substrate

a. Add 5 μL of coenzyme I application solution to sample wells.

b. Mix fully and incubate at 37 ℃ for 15 min.

c. Add 25 μL of chromogenic agent to each well. Mix fully and incubate at 37 ℃ for 15 min.

d. Add 200 μL of alkali reagent application solution to each well.

e. Mix fully and stand at room temperature for 5 min.

f. Measure the OD values of each well with microplate reader at 450 nm

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Serum (plasma) and other liquid sample:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1 L of sample at 37℃ for 15 minutes in the reaction system is defined as 1 unit.

$$\text{LDH activity (U/L)} = (\Delta A_{450} - b) \div a \times f \times 1000^*$$

Tissue and cells sample:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1 g protein at 37℃ for 15 minutes in the reaction system is defined as 1 unit.

$$\text{LDH activity (U/gprot)} = (\Delta A_{450} - b) \div a \times f \div C_{pr} \times 1000^*$$

[Note]

y: The absolute OD value of standard;

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA_{450}: $OD_{Sample} - OD_{Control.}$

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample (gprot/L)

1000*: 1 L=1000 mL

To easily calculate the test results, refer to the calculation file available on the webpage.

Example analysis

For Human serum, dilute human serum with PBS (0.01 M, pH 7.4) for 10 times, take 0.02 mL of diluted sample and carry the assay according to the operation steps. The results are as follows:

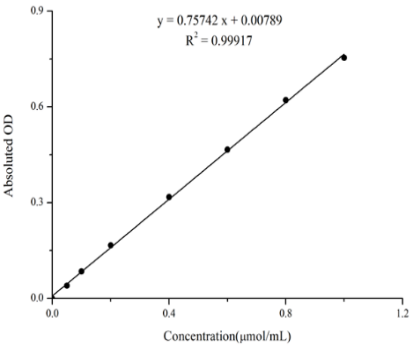
standard curve: y = 0.75742 x +0.00789, the average OD value of the sample is 0.232, the average OD value of the control is 0.115, and the calculation result is:

$$\text{LDH activity (U/L)} = (0.232 - 0.115 - 0.00789) \div 0.75742 \times 10 \times 1000 = 1440.548 \text{ U/L}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–1 µmol/mL standard.

Concentration (µmol/mL)	OD at 450 nm	Standard Curve
1.00	0.844	
0.80	0.712	
0.60	0.557	
0.40	0.408	
0.20	0.257	
0.10	0.175	
0.05	0.131	
0.00	0.091	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	14.50	250.00	662.00
%CV	2.3	1.6	1.5

CV = Coefficient of Variation

- Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	14.50	250.00	662.00
%CV	2.2	2.4	2.6

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples at dilutions from 1:10 to 1:60 in Assay Buffer

Sample Type	Range (**U/L**)	Average (**U/L**)
Human serum	1211–1691.586	1440.548
Human plasma	1.026–1.212	1598.51

▪ Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/mL)	0.08	0.24	0.76
Observed Conc. (μmol/mL)	0.1	0.2	0.7
Recovery rate (%)	98	99	97

▪ Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'
[Note]: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells												

- Sensitivity

The analytical sensitivity of the assay is 6 U/L LDH. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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