ELK Biotechnology
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Lactate Dehydrogenase (LDH) Assay Kit

CAT/NO.: BC092 Microplate Method

1. Reagent Composition and Preparation

	Component	Format:96T	Format:48T	Preservation
Reagent I	Base Buffer	1Bottle×5ml	1Bottle×3ml	2～8℃
Reagent II	Coenzyme I	Powder	Powder	Frozen at-20℃
	Preparation of Coenzyme I Solution: Add 1.3ml double distilled water (DDW) to dissolve the powder and the solution can be stored for 2 week when frozen and it is recommended to store in different containers to avoid multiple freezing and thawing			
Reagent III	2,4-dinitrophenylhydrazine	1 Bottle×5ml	1 Bottle×3ml	2～8℃ without light struck
Reagent IV	4M NaOH	1 Bottle×5ml	1 Bottle×3ml	2～8℃
	Preparation of 0.4M NaOH Solution : Dilute the solution with DDW to 10 times the initial solution volume and prepare exact amount needed.			
Reagent V	2mM Pyruvic Acid Solution	1 Bottle×1ml	1 Bottle×1ml	2～8℃
	0.2mM Standard Pyruvic Acid Solution: Dilute the solution with DDW to 10 times the initial solution volume and prepare exact amount needed.			

2. Sample Collection and Preservation

I. This assay kit is designed for the activity of LDH in animal serum, tissues, perfusate and cell culture.

II. Gather samples with conventional method and can be serum, plasma, supernatant from cell culture, tissues and cells.

III. Samples should be preserved at -20℃ if unfinished.

3. Measurement Procedure

Compositionsμl	Blank	Standard	Sample	Reference
DDW	20	4		4
0.2mM Pyruvic Acid		16		
Sample			16	16
Base Buffer	20	20	20	20
Coenzyme I			4	
Mix and warm at 37℃ for 15min				
2,4-dinitrophenylhydrazine	20	20	20	20
Mix and warm at 37℃ for 15min				
0.4MNaOH Solution	200	200	200	200

Mix and set aside at room temperature for 5 min. Regulate the microplate reader at 450nm to record the absorbed optical density (OD).

Sample Dosage Reference: 5-20μl 0.01% mouse brain tissue homogenate. 10-30μl 10 times diluted human serum. The samples can be diluted with Physiological saline if the activity result is too high.

Note: No coenzyme I solution should be added into the reference tube.

Note: The sequence of addition shall follow the table and addition of coenzyme I before adding base buffer is prohibited.

4. Calculation Formula and Example

I. LDH Activity Definition and Calculation Formula for Serum Samples

Definition: Activity unit is defined as number of μmols pyruvic acid generated in the reaction system with 1L serum at 37° C while the reaction period is a quarter.

Calculation Formula

$$\underset{U/L}{LDH\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times C_{standard}(0.2mM) \times N \times 1000$$

N:Sample dilution before test;
1000: Unit conversion,mL→L;

II. LDH Activity Definition and Calculation Formula for Tissue Samples

Definition: Activity unit is defined as number of μmols pyruvic acid generated in the reaction system with 1g protein in the tissue at 37° C while the reaction period is a quarter.

Formula

$$\underset{U/L}{LDH\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{OD_{Standard} - OD_{Blank}} \times C_{standard}(0.2mM) \div C_{protein}\ (gprot/ml)$$

III. Example for Serum Sample

1. LDH activity is measured from 20μl 10 times diluted human serum and the OD values for blank, standard, sample and reference tubes are 0.0675, 0.2464, 0.2383 and 0.1173 respectively.

$$\underset{U/L}{LDH\ Activity} = \frac{0.2383 - 0.1173}{0.2463 - 0.0675} \times 0.2mM \times 1000 \times 10 = 1353.47(U/L)$$

2. LDH activity is measured from 20μl 50 times diluted rat serum and the OD values for blank, standard, sample and reference tubes are 0.0675, 0.2463, 0.3043 and 0.0710 respectively.

$$\underset{U/L}{LDH\ Activity} = \frac{0.3043 - 0.0710}{0.2463 - 0.0675} \times 0.2mM \times 1000 \times 50 = 13048.1(U/L)$$

IV. Example for Tissue Sample

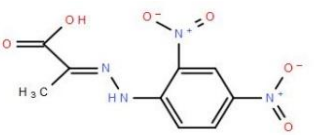
1. LDH activity is measured from 20μl 0.02% rat kidney tissue homogenate and the OD

values are 0.0675, 0.2464, 0.2460 and 0.0649 respectively. Protein content for 1% rat kidney tissue homogenate is 1.535mg/ml

$$\underset{U/L}{LDH\ Activity} = \frac{0.2460 - 0.0649}{0.2464 - 0.0675} \times 0.2mM \div (1.535 \times 10^{-3} \times 0.02) = 6599.35(U/L)$$

5. Principle of Measurement

Lactic Acid $\xrightarrow{LDH}$ Pyruvic Acid

Pyruvic Acid + 2,4-dinitrophenylhydrazine $\xrightarrow[Alkaline]{37℃}$ 

The generated compound is light chocolate color and according to Beer-Lambert's Law, the activity can be determined.

6. Note

1. Please be considerate as the amount added is small. It is recommended to hold the pipette with two hands. Also, please ensure the least amount of reagent remained in the pipette and additionally centrifuge at low speed to fully mix the reagent in order to achieve smaller error.
2. Please add the reagent softly to avoid splitting.
3. Shake the microplate moderately to avoid splitting or insufficient mixing. Also, please shake down the liquid on the wall and then shake horizontally.
4. Please measure the blank absorbance for the microplate before measurement and subtract the blank absorbance for a reliable result.
5. Because of the small amount of coenzyme I added, please
6. Avoid bubble generation for sample prepare and break the bubbles formed before measurement.
7. This assay kit is designed strictly for scientific research

Appendix I Standard Curve Establishment

1. Pretreatment

Dilute the 2mM pyruvic acid solution with DDW to 200,100, 50, 20, 10, 5 and 2 times the initial volume respectively to calibrate the standard curve.

2. Measurement

Compositionsμl	Blank	Standard
DDW	20	4
0.2mM Pyruvic Acid		16
Base Buffer	20	20
Mix and warm at 37℃ for 15min		
2,4-dinitrophenylhydrazine	20	20
Mix and warm at 37℃ for 15min		
0.4MNaOH Solution	200	200

Mix and set aside at room temperature for 5 min. Regulate the microplate reader at 450nm to record the absorbed optical density (OD).

Appendix II Search for Best Rat Serum LDH Concentration

I. Reagent and Preparation

As mentioned above

II. Sample Source

The rat serum provided by Nanjing Jiancheng Bioengineering Institute.

III. Measurement

Compositionsμl	Blank	Standard	Sample	Reference
DDW	20	4		4
0.2mM Pyruvic Acid		16		
Sample			16	16
Base Buffer	20	20	20	20
Coenzyme I			4	
Mix and warm at 37℃ for 15min				
2,4-dinitrophenylhydrazine	20	20	20	20
Mix and warm at 37℃ for 15min				
0.4MNaOH Solution	200	200	200	200

Mix and set aside at room temperature for 5 min. Regulate the microplate reader at 450nm to record the absorbed optical density (OD).

IV. Results

Coefficient of Dilute	Blank	100	50	20	10	5
Sample OD	0.0589	0.2799	0.3973	0.5795	0.6929	0.7427
Reference OD	0.0589	0.0613	0.0667	0.0851	0.1064	0.1509
OD Difference	0.0000	0.2186	0.3306	0.4944	0.5866	0.5918

Appendix III Search for Best Rat Kidney Homogenate Concentration for LDH Measurement

I. Reagent and Preparation

As mentioned above

II. Sample Source

The rat serum provided by Nanjing Jiancheng Bioengineering Institute.

III. Pre-treatment

10% homogenate supernatant was prepared by homogenizing rat kidney tissue with physiological saline and then diluted to 1%. Prepare 0.01%, 0.02%, 0.05%, 0.1%, 0.2% and 0.5% solution respectively with 1% solution.

Also, measure the total amount of protein with 0.5% homogenate by the Coomassie Brilliant Blue Method.

IV. Measurement

Compositionsμl	Blank	Standard	Sample	Reference
DDW	20	4		4
0.2mM Pyruvic Acid		16		
Sample			16	16
Base Buffer	20	20	20	20
Coenzyme I			4	
Mix and warm at 37℃ for 15min				
2,4-dinitrophenylhydrazine	20	20	20	20
Mix and warm at 37℃ for 15min				
0.4MNaOH Solution	200	200	200	200

Mix and set aside at room temperature for 5 min. Regulate the microplate reader at 450nm to record the absorbed optical density (OD).

V. Results

Coefficient of Dilute	Blank	100	50	20	10	5	2	1

Sample OD	0.0588	0.1653	0.2807	0.4473	0.6187	0.6979	0.8065	0.9133
Reference OD	0.0588	0.0594	0.0636	0.0833	0.0930	0.1340	0.2120	0.2835
OD Difference	0.0000	0.1059	0.2171	0.3641	0.5257	0.5639	0.5945	0.6298