GENLISA™ Lactate Dehydrogenase (LDH) Assay

REF : KBCA1691

Ver 1.1

RUO

Biochemical Assay for the Quantitative Determination of Lactate Dehydrogenase (LDH) in serum, plasma tissue cells, cell culture supernatants and other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA1691 Σ 96 tests

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Introduction:

The GENLISA™ Assay kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma, tissue cells and cell culture supernatant as validated with the kit. The kit employs in vitro quantitative determination of Lactate Dehydrogenase in the sample.

Intended Use:

The GENLISA™ Lactate Dehydrogenase (LDH) Assay kit is used as an analytical tool for quantitative determination of Lactate Dehydrogenase (LDH) in serum, plasma, tissue cells, cell culture supernatants and other biological samples.

Principle:

The optical spectrum absorbance at certain wavelength is proportion to the concentration of Quinone compound in the solution. This concentration relates to the Triglyceride content of the sample. Thus the Triglyceride's content can be determined via the absorbance difference between the standard tube and the sample tube according to the absorbance of the blank tube.

Materials Provided:

1. Reagent 1 (Base Buffer) – 1 vial x 5 ml
2. Reagent 2 (Coenzyme I, Lyophilized) – 1 vial
3. Reagent 3 (2,4-dinitrophenylhydrazine) – 1 vial x 5 ml
4. Reagent 4 (4M NaOH) – 1 vial x 5 ml
5. Reagent 5 (2mM Pyruvic Acid Solution) – 1 vial x 1 ml
6. Microwell Plate (96 wells) - 1 no.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. Standard should be stored at-20°C.
3. All the reagents should be used within 12 months from manufacturing date.
4. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

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.

Assay Procedure

Composition (ul)	Blank	Standard	Sample	Reference
DDW / Purified Water	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 well.
Incubate at room temperature for 5 min.
Read the absorbance at 450nm.

Sample Dosage Reference:
5 - 20 ul 0.01% mouse brain tissue homogenate. 10 - 30 ul 10 times diluted human serum. The samples can be diluted with physiological saline if the activity result is too high.
Note:
i) No coenzyme I solution should be added into the reference tube.
ii) The sequence of addition shall follow the table and addition of coenzyme I before adding base buffer is prohibited.

Interpretation of Results

I. LDH Activity Definition and Calculation Formula for Serum Samples

Definition: Activity unit is defined as number of umols pyruvic acid generated in the reaction system with 1 lt 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 umols pyruvic acid generated in the reaction system with 1 gm 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

a) LDH activity is measured from 20 ul, 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.

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

b) LDH activity is measured from 20 ul, 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

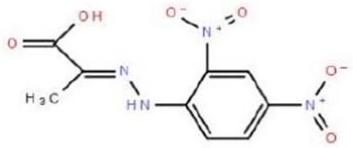
LDH activity is measured from 20 ul, 0.02% rat kidney tissue homogenate and the OD values are 0.0675, 0.2464, 0.2460 and 0.0649 respectively. Protein content for 1% rat kidney tissue homogenate is 1.535 mg/ml.

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

Principle of Measurement

$$\text{Lactic Acid} \xrightarrow{LDH} \text{Pyruvic Acid}$$

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



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

Note:

1. Run the assay carefully for pipetting and incubation to avid experimental errors.
2. Shake the microplate moderately and well.
3. Measure the blank absorbance for the microplate and subtract the blank absorbance for a reliable result.

Appendix I Standard Curve Establishment

1. **Sample Pretreatment**

Dilute the 2 mM 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. Assay Procedure

Compositions ul	Blank	Standard
DDW	20	4
0.2 mM Pyruvic Acid		16
Base Buffer	20	20
Mix and warm at 37°C for 15 min		
2,4-dinitrophenylhydrazine	20	20
Mix well. Incubate at 37°C for 15 min		
0.4MNaOH Solution	200	200

Mix well.
Incubate at room temperature for 5 min.
Read the absorbance at 450 nm.

Appendix II Rat Serum LDH Concentration

1. Sample Pretreatement
As above

2. Assay Procedure

Compositions ul	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 well. Incubate at 37°C for 15 min				
2,4-dinitrophenylhydrazine	20	20	20	20
Mix well. Incubate at 37°C for 15 min				
0.4MNaOH Solution	200	200	200	200

Mix well.
Incubate at room temperature for 5 min.
Read the abosorbance at 450 nm.

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 Rat Kidney Homogenate LDH Concentration

1. Sample Pretreatment

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.

2. Assay Procedure

Compositions ul	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°C for 15 min				
2,4-dinitrophenylhydrazine	20	20	20	20
Mix and warm at 37°C for 15 min				
0.4MNaOH Solution	200	200	200	200

Mix well.
Incubate at room temperature for 5 min.
Read the abosorbance at 450 nm.

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

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

Negative Blank

Blank tube absorbance value should be ≤0.200.

Assay Concentration Range
Concentration range is 0 - 9.04 mM with r^2 >0.99.
Note: The absorbance value A is around 0.22 - 0.29 for the 2.26mM Standard solution.

Precision
Inter and Intra Assay ≤ 10%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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