Lactate Dehydrogenase (LDH) GENLISA™ Assay

REF : KBCA1696

Ver 1.0

RUO

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 KBCA1696 **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 Lactate Dehydrogenase (LDH) GENLISA™ 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.

$$\text{Triglyceride} + H_2O \xrightarrow{\textit{Lipase}} \textit{Glycerol} + \textit{Fatty Acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\textit{Glycerol Kinase}} \textit{Glycerol} - 3 - \textit{Phosphate} + \textit{ADP}$$

$$\text{Glycerol} - 3 - \text{Phosphate} + O_2 \xrightarrow{\textit{Glycerol-3-Phosphate Oxidase}} \textit{Dihydroxyacetone Phosphate} + H_2O_2$$

$$H_2O_2 + 4 - \text{AAP} + \text{p} - \text{Chlorophenol} \xrightarrow{\textit{Peroxidase}} \text{Quinone (Red Compounds)}$$

Materials Provided:

1. Flat-Bottom Microtiter Plate – 96 wells
2. Enzyme Reagent – 25 ml
3. Standard Solution – 1 ml

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 Preparation:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Sample Pre-Treatment

1. Serum (Plasma): Can be used for further measurement directly. The serum should be diluted with physiological saline if the concentration of triglyceride in serum exceeds the concentration range allowed.
2. Medium Sample: The medium should be extracted and separated by centrifugation at 2500rmp for 10 minutes. The supernatant is desired.Tissue Sample: The sample is weighed precisely and for every 1g of the sample, 9mL homogenate medium should be mixed in ice water bath. The homogenate treated with centrifugation at 2500rpm for 10 minutes would be separated and supernatant is extracted for further use.
   Note 1.The homogenate medium is 0.1mM phosphate buffer (pH=7.4) or physiological saline for tissue samples without high fat part.
   Note 2. For high fat or partial high fat sample, the homogenate medium used is ethyl alcohol absolute.
3. Sample Cells
   Cell Harvesting
   Cell suspension is extracted and centrifuged with 1000rpm for 10 minutes. Supernatant should be discarded and isotonic buffer (0.1M, pH 7-7.4 phosphate buffer recommended) is used to wash the precipitates for once or twice. The suspension is separated by centrifugation for 10 minutes and theprecipitates should be kept for further treatment.
   Cell Disruption
   0.2 to 0.3 mL homogenate medium should be added and homogenized (0.1M, pH 7-7.4 phosphate buffer or physiological saline recommended). Cells can be disrupted with ultrasonic disruptor in ice water bath. The ultrasonic disruptor is 300W and the disruption is repeated for 3-5 times with the period of 3-5 seconds and interval of 30 seconds. The homogenate can be measured directly. Also, Lysate can be used directly for the later measurement (Triton X-100, 1-2% recommended for the splitting period of 30-40 minutes).
   Note that the cell density in the sample is recommended to be no less than 1 million/mL. Microscope can be used to observe the completeness of cell disruption.

Assay Procedure:

Operated in 96-well Microtiter Plate with Microplate Reader

1. Take 3 eppendorf tubes, label them as Blank, standard and sample.
2. Add 2.5 µL distilled water in the blank tube, 2.5 µL of 2.26mM Standard Solution in the standard tube and 2.5 µL sample in the sample tube.
3. Add 250 µL Enzyme Reagent in each tube and mix well
4. Incubate the tube at 37 ^{0}C for 10 mins.
5. Read the absorbance at 500 nm

Operated by Automatic Biochemical Analyzer

1. Take 2.5µL of sample & 250µL of enzyme reagent and mix well
2. Incubate the tube at 37 ^{0}C for 10 mins.
3. Adjust the analyzer with enzyme reagent and distilled water, then measure the absorbance A.
4. Read the absorbance at 510 nm using the End-Point method with the reaction proceeding in the forward direction.

Calculation of Results:

For serum or other liquid sample (For Microplate Reader)

$$\underset{mM}{Triglyceride\ Concentration} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{(2.26mM)}{C_{Standard}}$$

For serum or other liquid sample (For Automatic Biochemical Analyzer)

$$\underset{mM}{Triglyceride\ Concentration} = \frac{A_{sample}}{A_{Standard}} \times \underset{(2.26mM)}{C_{Standard}}$$

Example: 2.5mL human serum was measured and the absorbance values are 0.0573, 0.2340 and 0.1283 for blank, standard and sample solution. The Triglyceride concentration is shown below

$$\underset{mM}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \underset{(2.26mM)}{C_{Standard}} = \frac{0.1283 - 0.0573}{0.2340 - 0.0573} \times 2.26mM = 0.9081\text{mM}$$

Example: 2.5µL blood plasma was measured and the absorbance values are 0.0573, 0.2340 and 0.1030 in accordance to the sequence listed above.

$$\underset{Mm}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \underset{(2.26mM)}{C_{Standard}} == \frac{0.1030 - 0.0573}{0.2340 - 0.0573} \times 2.26mM = 0.5845\text{mM}$$

Formula for Samples of Tissue or Cell

For Microplate Reader

$$\underset{mmol/g}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \underset{(2.26mM)}{C_{Standard}} \div C(ggprot/L)$$

For Automatic Biochemical Analyzer

$$\underset{mmol/g}{Triglyceride} = \frac{A_{sample}}{A_{Standard}} \times \underset{(2.26mM)}{C_{Standard}} \div C(gprot/L)$$

Example: 2.5 homogenate from mouse's liver was measured and the absorbance values are 0.0580, 0.2352 and 0.1583 in accordance to the sequence listed above. Also, the protein concentration is measured to be 12.0121 g/L

$$\underset{mmol/gprot}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \underset{(2.26mM)}{C_{Standard}} \div \rho(gprot/L)$$

$$= \frac{0.1583 - 0.0580}{0.2352 - 0.0580} \times 2.26 \div 12.0121 = 0.1065mmol/g$$

Quality Control: 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:

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1. Blank tube absorbance value≤0.200.
2. Concentration range 0-9.04mM with r^2>0.995.
3. Accuracy with the relative deviation≤10%
4. The absorbance value A is around 0.2200-0.2900 for the 2.26mM Standard solution.
5. The precision of the measurement is ≤5.5% and coefficient of variation≤ 8.0%.
6. The reagent in the original package should be preserved at 2-8℃ without light-struck and is stable within 12 months. The unsealed package can be preserved for one month in the same environment.

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 Rat 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.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.1 Standard No.1			
1B 2B	Standard No.2 Standard No.2			
1C 2C	Standard No.3 Standard No.3			
1D 2D	Standard No.4 Standard No.4			
1E 2E	Standard No.5 Standard No.5			
1F 2F	Standard No.6 Standard No.6			
1G 2G	Standard No.7 Standard No.7			
1H 2H	Standard No.8 Standard No.8			
3A 4A	Sample			
3B 4B	Sample			

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SYMBOLS KEY

MTP	Coated Microtiter Plate (96 wells)
STD	Standard
STD | DIL	Standard Diluent
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature