Lactate Dehydrogenase Assay Kit

Lactate Dehydrogenase Assay Kit is a detection kit for the quantification of Lactate Dehydrogenase activity in serum, plasma, and cell/tissue extract samples.

Catalog number: ARG81352

Package: 100 assay

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INTRODUCTION

Lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells (animals, plants, and prokaryotes). LDH catalyzes the conversion of lactate to pyruvate and back, as it converts NAD+ to NADH and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another.

LDH is expressed extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease such as heart failure.

On blood tests, an elevated level of lactate dehydrogenase usually indicates tissue damage, which has multiple potential causes, reflecting its widespread tissue distribution:

Hemolytic anemia, vitamin B12 deficiency anemia; infections such as infectious mononucleosis; meningitis, encephalitis, HIV/AIDS. (It is notably increased in sepsis); Infarction, such as bowel infarction, myocardial infarction and lung infarction; Acute kidney disease; Acute liver disease; Rhabdomyolysis; Pancreatitis; Bone fractures; Cancers, notably testicular cancer and lymphoma. (A high LDH after chemotherapy indicates a poorer response and a worse survival rate); Severe shock; Hypoxia.

Low and normal levels of LDH do not usually indicate any pathology. Low levels may be caused by large intake of vitamin C.

LDH is a protein that normally appears throughout the body in small amounts. [Wikipedia: Lactate dehydrogenase]

PRINCIPLE OF THE ASSAY

This Lactate Dehydrogenase Assay Kit is simple and could be used to quantify LDH activity from serum, plasma, and cell/tissue extract samples. In this Lactate Dehydrogenase Assay Kit, LDH catalyzes the conversion of lactate to pyruvate, as it converts NAD+ to NADH. The produced NADH could be used to reduce a tetrazolium salts, MTT through diaphorase reaction. The reduced form MTT is purple color and it can be detected by absorbance at 565 nm. The intensity of the purple color formed is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Substrate Buffer	20 ml (ready to use)	-20°C
NAD Solution	1 ml (ready to use)	-20°C
MTT Solution	1.5 ml (ready to use)	-20°C
Diaphorase	120 µl (ready to use)	-20°C
Standard	1.5 ml (ready to use)	-20°C

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 565 nm
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Multi-channel pipettes
- Heat block or hot water bath or oven
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -20°C at all times.
- Assays can be executed at room temperature or 30°C.
- All components should be equilibrated to room temperature or 30°C and briefly centrifuge all reagent before opening.
- Mix well all reagent and briefly spin down the reagents before use.
- This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. All samples should be clear and free of any turbidity or particles. Liquid samples (e.g. non-hemolyzed serum, plasma) can be assayed directly.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot and store samples at ≤ -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using Citrate or heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

Tissue lysate: Rinse tissue in phosphate buffered saline (pH 7.4) to remove blood prior to dissection. Homogenize tissue in 5 mL buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA, per gram tissue and then centrifuge at 10,000 x g for 15 min at 4°C. Collect supernatant for assay. Assay immediately or aliquot and store samples at ≤ -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

Cell lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of

cold buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA. Centrifuge at 10,000 x g for 15 min at 4°C. Collect supernatant for assay. Assay immediately or aliquot and store samples at ≤ -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate Buffer, Diaphorase, NAD Solution, MTT Solution and Standard:** All reagents are ready to use, mix it well by vigorous shaking and centrifuge reagent tubes before use. Equilibrate all reagents to desired reaction temperature (e.g. 25°C or 30°C) before use.
- **Sample**: For unknown samples or if samples might contain Lactate Dehydrogenase activity higher than highest linear range of 200 IU/L, the samples are suggested to perform several dilutions with distilled water to ensure that LDH activity is within the linear range of 2 to 200 IU/L. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay. (Note: 3 µl of serum/plasma sample may be sufficient for the assay.)

  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Sample Working Reagent:** *Prepare before use,* mix 14 µl of MTT Solution, 8 µl of NAD Solution, 1 µl of Diaphorase and 175 µl of Substrate Buffer for each well. Add 190 µL of Working Reagent quickly to each sample wells. Tap plate to mix.

ASSAY PROCEDURE

All components should be equilibrated to room temperature (or 30°C) and briefly mix and centrifuge reagent tubes before use. Standards and samples should be assayed in at least duplicates.

1. Add **200 µl** of **distilled water** and **200 µl** of **Standard** into separate wells of the clear flat bottomed 96-well microplate as background control and standard wells.
2. Add **10 µl** of **each samples** into the corresponding wells.
3. Add **190 µl** of **Sample Working Reagent** into each sample well. Tap the plate to mix it well immediately.
4. **Read O.D.** with a microplate reader at **565 nm** immediately **(time zero, OD_0)**. Incubate the plate at room temperature (or 30°C). And **read O.D. again at 25 min (time 25 min, OD_{25}).**

Summary of Assay Procedure

Reagent	Sample	Standard	Background control
Sample	10 µl	-	-
Standard	-	200 µl	-
Distilled water	-	-	200 µl
Working Reagent	190 µl	-	-
Gently tap plate to mix thoroughly.			
Read O.D. with a microplate reader at 565 nm immediately.			
OD label	$OD_{Sample-0}$	$OD_{Standard}$	OD_{H2O}
Incubate the plate at room temperature (or 30°C)			
Read O.D. with a microplate reader at 565 nm again at 25 min.			
OD label	$OD_{Sample-25}$	-	-

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. LDH activity can then be calculated as follows:

   LDH Activity (IU/L)=

   N X [($OD_{Sample-25}$ – $OD_{Sample-0}$) / (ε_{MTT} X /)] X [(Reaction Vol) / (Time X Sample Vol)]

   = N X 43.68 X [($OD_{Sample-25}$ – $OD_{Sample-0}$) / ($OD_{standard}$ – OD_{H2O})]

   Note:

   $OD_{Sample-0}$ = OD_{565nm} of sample read at 0 min

   $OD_{Sample-25}$ = OD_{565nm} of sample read at 25 min

   $OD_{standard}$ = OD_{565nm} of Standard read at 0 min

   OD_{H2O}= OD_{565nm} of Standard read at 0 min

   ε_{MTT} = molar absorption coefficient of reduced MTT

   / = light path length which is calculated from the calibrator.

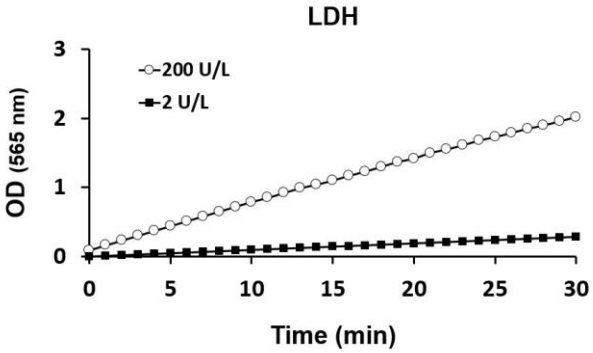
   Reaction Vol = 200 µL

   Sample Vol = 10 µL

   N = N is the dilution factor (if sample has been diluted)
3. Unit definition: 1 Unit (IU) of LDH will catalyze the conversion of 1 µmole of lactate to pyruvate per min at pH 8.2.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Lactate Dehydrogenase Assay Kit was 2 IU/L. The Linear detection range ranged from 2 - 200 IU/L (for 10 µl of samples).

Example of result

Samples were assayed using the 96-well plate protocol. The LDH activity (IU/L) was 41 for a human serum, 220 for rat serum and 88 for fetal bovine serum, respectively.