ab197000
Lactate Dehydrogenase (LDH) Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Lactate Dehydrogenase in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 4, Last Updated 24 November 2023

Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 3

GENERAL INFORMATION

3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

ASSAY PREPARATION

9. REAGENT PREPARATION 8
10. STANDARD PREPARATION 9
11. SAMPLE PREPARATION 10

ASSAY PROCEDURE and DETECTION

12. ASSAY PROCEDURE and DETECTION 12

DATA ANALYSIS

13. CALCULATIONS 14
14. TYPICAL DATA 16

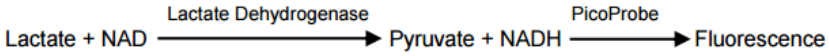
RESOURCES

15. QUICK ASSAY PROCEDURE 18
16. TROUBLESHOOTING 19
17. FAQ 21
18. INTERFERENCES 22
19. NOTES 23

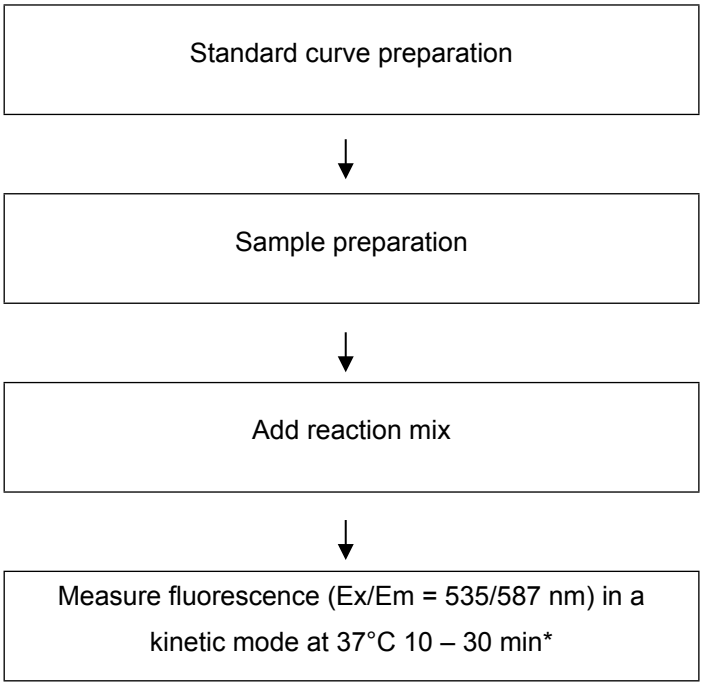
1. BACKGROUND

Lactate Dehydrogenase (LDH) Assay Kit (fluorometric) (ab197000) provides a quick and easy method for monitoring Lactate Dehydrogenase (LDH) activity in a wide variety of samples. In this assay, Lactate Dehydrogenase converts lactate into pyruvate and NADH, which reacts with the specific fluorescent probe to generate an intense fluorescent product (Ex/Em = 535/587 nm). This kit is simple, highly sensitive and high-throughput adaptable and can detect Lactate Dehydrogenase activity as low as 1 µU/mL.

Lactate dehydrogenase (LDH, L-Lactate NAD oxidoreductase, EC 1.1.1.27) is an ubiquitous enzymes among vertebrate organisms which catalyzes the reversible conversion of pyruvate to lactate, with concomitant conversion of NADH and NAD+. LDH is cytoplasmic in its cellular location and in any one tissue is composed of one or two of five possible isoenzymes. During tissue damage, LDH is released into the bloodstream; therefore it serves as a marker for various diseases and common injuries.



2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
LDH Assay Buffer	110 mL	-20°C	4°C / -20°C
Developer Mix I/LDH Substrate Mix (lyophilized)	1 vial	-20°C	-20°C
PicoProbe (1.5 ml)/PicoProbe (in DMSO)	1.4 mL	-20°C	-20°C
NADH Standard I/NADH Standard (lyophilized)	1 vial	-20°C	-20°C
LDH Positive Control	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 535/587 nm
- 96 well plate: white plates (flat bottoms) for fluorometric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **LDH Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 **Developer Mix I/LDH Substrate Mix:**

Reconstitute in 1.1 mL ddH_2O. Pipette up and down to dissolve completely. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.3 **PicoProbe (1.5 ml)/PicoProbe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light and moisture. Once the probe is thawed, use with two months. Keep on ice while in use.

9.4 **NADH Standard I/NADH Standard:**

Reconstitute the NADH Standard I/NADH Standard in 500 µL of ddH_2O to generate a 1 mM (1 nmol/µL) standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **LDH Positive Control:**

Reconstitute with 100 µL LDH Assay Buffer. Aliquot reconstituted positive control so that you have enough volume to perform the desired number of tests. Store at -20°C.

Prior to use, dilute 1:100 in LDH Assay Buffer and mix. Use immediately. Keep on ice while in use. Do not store unused diluted positive control.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare a 50 µM NADH standard by diluting 50 µL of the reconstituted NADH Standard I/1 mM NADH standard with 950 µL of ddH_2O.

10.2 Using 50 µM NADH standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc. NADH in well
1	0	150	50	0 pmol/well
2	6	144	50	100 pmol/well
3	12	138	50	200 pmol/well
4	18	132	50	300 pmol/well
5	24	126	50	400 pmol/well
6	30	120	50	500 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold LDH Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Keep on ice for 10 minutes.

11.1.6 Centrifuge sample for 5 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a clean tube.

11.1.8 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold LDH Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Keep on ice for 10 minutes.

11.2.6 Centrifuge samples for 5 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.2.7 Collect supernatant and transfer to a clean tube.

11.2.8 Keep on ice.

11.3 **Plasma, Serum and Urine and other biological fluids:**

Serum and urine samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

11.4 **Cell culture and fermentation media:**

Cell culture and fermentation media can be tested directly by adding sample to the microplate wells.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with LDH Assay Buffer).
- Positive control = 2 – 10 µL 1:100 diluted Positive control (Section 9.5) (adjust volume to 50 µL/well with Assay Buffer).

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)
LDH Assay Buffer	**45.5**
PicoProbe (1.5 ml)/PicoProbe	**2.5**
Developer Mix I/LDH Substrate Mix	**2**

Mix enough reagents for the number of assays (samples, standards and positive control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + Standards + positive control + 1).

12.3 Add 50 µL of Reaction Mix into each standard, sample and positive control wells.

12.4 Measure fluorescence immediately at Ex/Em = 535/587 nm in a kinetic mode, every 2 – 3 minutes, for 10 – 30 minutes at 37°C.

NOTE: *Sample incubation time can vary depending on LDH activity in the samples. We recommend measuring fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) in the linear range to calculate the LDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).*

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of LDH.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Activity of LDH is calculated as:

$\mathbf{\Delta RFU_{535/587} = RFU2 - RFU1}$

Where:

RFU1 is the same reading at T1

RFU2 is the sample reading at T2

13.6 Use the ΔRFU to obtain B nmol of NADH generated by LDH during the reaction time ($\Delta T = T_2 - T_1$).

13.7 LDH activity (in pmol/min/µL or µU/µL or U/L) in the test samples is calculated as:

$$LDH\ Activity = \left(\frac{B}{\Delta T\ x\ V}\right) * D$$

Where:

B = NADH amount generated between T1 and T2 (nmol).

ΔT = reaction time (T2 – T1) (min).

V = pretreated sample volume added into the reaction well (mL).

D = Sample dilution factor.

NADH molecular weight: 763.0 g/mol.

Unit definition: One unit of Lactate Dehydrogenase is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH 8.8 at 37°C.

LDH activity in samples can be expressed in mU/mg of protein.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

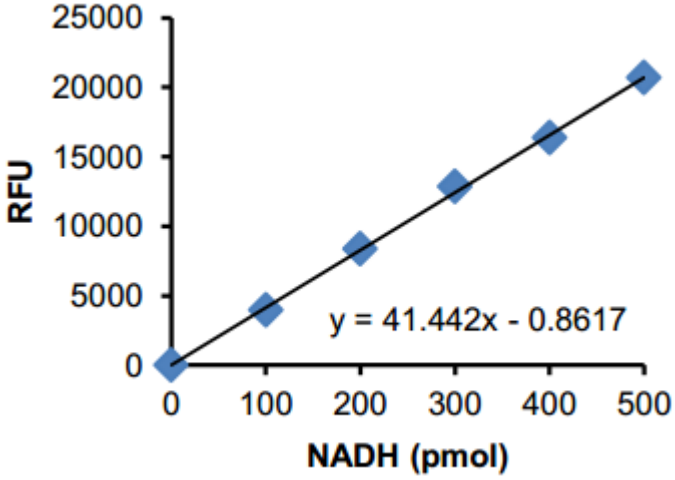


Figure 1. Typical NADH Standard calibration curve using fluorometric reading.

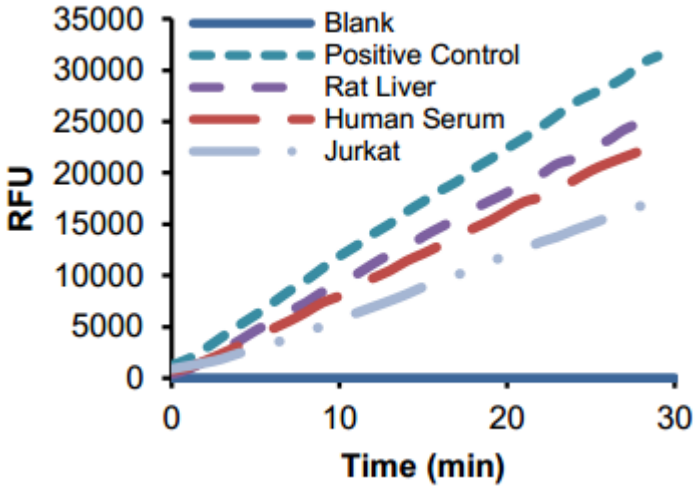


Figure 2: Kinetic measurement of Lactate Dehydrogenase activity in various samples.

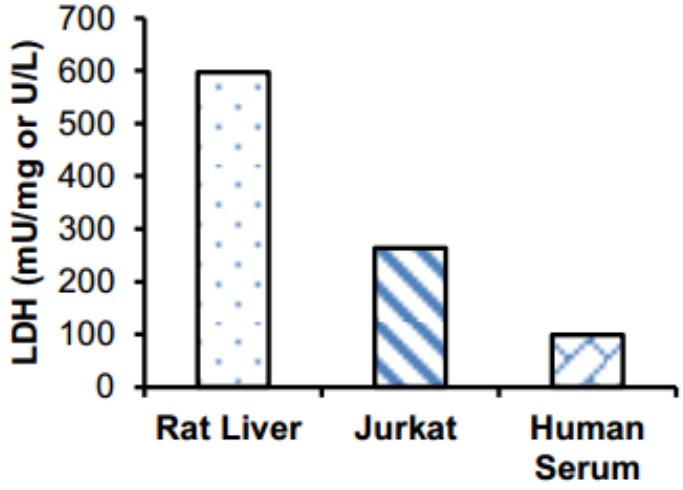


Figure 3: Relative LDH Activity was calculated in lysates prepared from rat liver (0.037 µg protein), Jurkat cells (0.053 µg protein), and human serum (0.2 µg protein).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, PicoProbe (1.5 ml)/PicoProbe, substrate and assay buffer; (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve reading).
- Set up plate for standard (50 µL), samples (50 µL) and diluted positive control wells (50 µL).
- Prepare Reaction Mix (Number samples + standards + 1).

Component	Fluorometric Reaction Mix (µL)
LDH Assay Buffer	**45.5**
PicoProbe (1.5 ml)/PicoProbe	**2.5**
Developer Mix I/LDH Substrate Mix	**2**

- Add 50 µL of Reaction Mix to the standard, sample and positive control wells.
- Measure fluorescence immediately at Ex/Em = 535/587 nm on a microplate reader on a kinetic mode at 37°C for 10 – 30 minutes.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. **FAQ**

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA: contains SDS which can destroy/decrease the activity of the enzyme.

19. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)