ab102526 Lactate Dehydrogenase (LDH) Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Lactate Dehydrogenase (LDH) in various samples. For research use only – not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab102526
(use abcam.cn/ab102526 for China, or abcam.co.jp/ab102526 for Japan)

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.

Materials Supplied:

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
LDH Assay Buffer	50 mL	-20°C	-20°C
Substrate Mix/LDH Substrate Mix (Lyophilized)	1 vial	-20°C	-20°C
NADH Standard I/NADH Standard (0.5 µmol; Lyophilized)	1 vial	-20°C	-20°C
LDH Positive Control/LDH Positive Control (Lyophilized)	1 vial	-20°C	-20°C

Storage and Stability:

Store kit at -20°C 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 before and after prep. Aliquot components in working volumes before storing at the recommended temperature. Reconstituted components are stable for 1 month.

Materials Required, Not Supplied:

- Microplate reader capable of measuring absorbance at OD 450 nm
- Double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- PBS

Reagent Preparation:

Briefly centrifuge small vials at low speed prior to opening.

1. **NADH Standard I/NADH Standard:**
   Reconstitute with 0.4 mL ddH_2O to generate a 1.25 mM NADH Standard Solution. Aliquot standard so that you have enough volume to perform the desired number of tests. Store aliquots at - 20°C. Keep on ice while in use. Use within 1 month.
2. **LDH Assay Buffer:**
   Ready to use as supplied. Warm to room temperature before use. Store at - 20°C.
3. **LDH Positive Control:**
   Reconstitute the whole vial with 200 µl LDH Assay Buffer. Aliquot the control so that you have enough volume to perform the desired number of tests. Store aliquots at - 20°C. Keep on ice while in use. Use within 1 month.
4. **Substrate Mix/LDH Substrate Mix:**
   Reconstitute in 1.1 mL ddH_2O by mixing solution for 10 minutes. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store aliquots at - 20°C. Use within 1 month.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

Using 1.25mM 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 (nmol/well)
1	0	125	50	0
2	5	120	50	2.5
3	10	115	50	5
4	15	110	50	7.5
5	20	105	50	10
6	25	100	50	12.5

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

Sample Preparation:

- 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, or if that is not possible, snap freeze samples in liquid nitrogen upon extraction and store at -80°C. When you are ready to test your samples, thaw them on ice. This can affect sample stability and readings can be lower than expected.

Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommendation = 1 – 2 x 10^6 cells).
2. Wash cells with cold PBS.
3. Homogenize cells on ice in 2 – 4 volumes of cold Assay Buffer.
4. Centrifuge cells at 4°C at 10,000 x *g* for 15 minutes in a cold microcentrifuge to remove any insoluble material.
5. Collect the supernatant, transfer to a new tube and keep on ice.

Tissue samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 100 mg tissue).
2. Wash tissue with cold PBS.
3. Homogenize tissue in 2 – 4 volumes of cold Assay Buffer using a Dounce homogenizer (10 – 50 passes) on ice.

4. Centrifuge samples at 4°C at 10,000 x *g* for 15 minutes in a cold microcentrifuge to remove any insoluble material.
5. Collect the supernatant, transfer to a clean tube and keep on ice.

Erythrocytes:

1. Harvest the amount of erythrocytes necessary for each assay (initial recommendation = 0.2 mL).
2. Wash tissue with cold PBS.
3. Homogenize cells on ice in 2 – 4 volumes of cold Assay Buffer.
4. Centrifuge cells at 4°C at 10,000 x *g* for 15 minutes in a cold microcentrifuge to remove any insoluble material.
5. Collect the supernatant, transfer to a new tube and keep on ice.

Serum, Urine and other fluid samples:

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.

Assay Procedure:

– Equilibrate all materials and prepared reagents to room temperature prior to use.
– We recommend that you assay all standards, controls and samples in duplicate.

Δ Note: Set up Sample Background Controls for colored samples, as the color may interfere with the reading.

1. Set up Reaction Wells

Standard wells = 50 µL standard dilutions
Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with LDH Assay Buffer).
Positive control = 2 – 5 µL Positive control (see step 3) (adjust volume to 50 µL/well with LDH Assay Buffer).

2. Reaction Mix

2.1 Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

- LDH Assay Buffer: 48 µL
- Substrate Mix/LDH Substrate Mix: 2 µL

2.2 Add 50 µL of Reaction Mix into each standard, sample and positive control sample wells. Mix well.
2.3 Measure output immediately at OD 450 nm (T1) on a microplate reader in a kinetic mode, every 2 – 3 minutes, for at least 30 – 60 minutes at 37°C protected from light.
Incubate reaction for up to 4 hours if LDH activity is low.

Δ Note: Incubation time depends on the LDH Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T1 and T2) in the linear range (OD values A1 and A2 respectively) to calculate the LDH activity of the samples.
For standard curve, do not subtract A2 from A1. Standard curve can also be read in end point mode (i.e. at the end of incubation time).

Calculations:

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of LDH activity.
4. Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Activity of LDH in the test samples is calculated as: $\Delta A_{450nm} = (A_2 - A_1)$
   Where A1 is the sample reading at time T1 and A2 is the sample reading at time T2.
   Use the ΔA_{450nm} to obtain B nmol of NADH generated by LDH during the reaction time ($\Delta T = T_2 - T_1$).
6. Activity of LDH in the test samples is calculated as:

$$LDH\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D = nmol/min/mL = mU/mL$$

   Where:
   B = Amount of NADH in sample well calculated from standard curve (nmol).
   ΔT = Reaction time (minutes).
   V = Original sample volume added into the reaction well (mL).
   D = Sample dilution factor.

NADH molecular weight = 763 g/mol
1 Unit LDH = amount of enzyme that catalyzes the conversion of lactate to pyruvate to generate 1.0 µmol of NADH per minute at pH8.8 at 37°C.

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

- FBS – if measuring LDH in cell culture medium

FAQs:

What is the detection range for this kit? The detection range is 1 -100 mU/mL of LDH.
Can this kit measure both intra- and extracellular LDH activity? This kit can measure both intra and extracellular Pyruvate. For intracellular measurement from cell samples follow the sample preparation step described in the protocol. For extracellular media, remove any cell debris by spinning down and optimize the volume needed to get values within the linear range of the standard curve.
How specific is this kit? It seems that this kit is only measuring the change between NAD+ and NADP, which can be catalyzed by enzymes other than LDH. Is there any way to be sure that the kit is measuring only LDH activity? The principle of the assay is the following:
LDH converts Lactate to pyruvate, generating NADH from NAD. The NADH interacts with the probe to generate color at 450 nm. The amount of colored product formed is directly proportional to the LDH activity in the sample. The buffer conditions (pH, presence of an electron transfer agent) are optimized for LDH activity and since the NADH generation is coupled to lactate conversion to pyruvate, this assay specifically detects LDH and no other NAD+ to NADH converting enzymes in the cell. Any nonspecific endogenous NADH can be accounted for by omitting the substrate in the reaction mix. This background value can be subtracted from the sample LDH activity value.
I want to measure LDH alpha (LDH5) activity, which converts pyruvate to lactate. Will this kit work for that purpose?
Lactate dehydrogenase catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. So LDH5 converts pyruvate to lactate and vice versa. In our assay, NAD+ (produced during pyruvate to lactate conversion by LDH5) is reduced to NADH, which interacts with a probe to produce a color (λmax = 450 nm). In essence, this assay can work for this objective. The reaction conditions and the relative amount of pyruvate and lactate decide which direction the reaction will go.

How can adherent cells be used for this assay?
Trypsinization can be used to detach cells. Then collect the cells, spin down, remove medium + trypsin, wash cells with PBS and then proceed with homogenization using the assay buffer in the kit. It is important to be careful since over-trypsinization might damage the plasma membrane and leak LDH into the medium.

Does phenol red in the media affect this assay?
Since only 2 – 50 µL sample are added per well, the color from phenol red is diluted by the assay buffer/reaction mix. Typically this does not affect the assay. The OD450 nm readings are in the yellow-brown range.

Technical Support

For all technical or commercial enquiries please go to:
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