ab102526 Lactate Dehydrogenase (LDH) Assay Kit (Colorimetric)

For 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:
http://www.abcam.com/ab102526 (use http://www.abcam.cn/ab102526 for China, or http://www.abcam.co.jp/ab102526 for Japan)

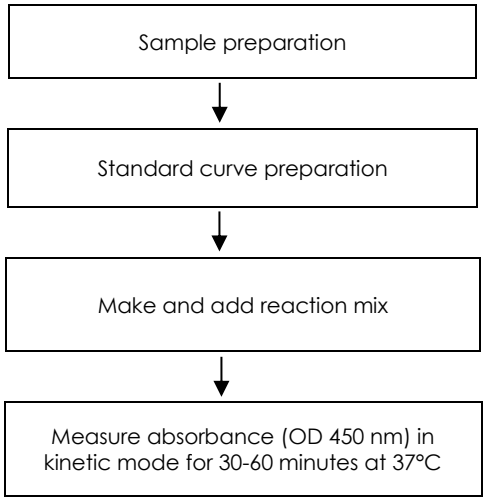
Background:

Lactate Dehydrogenase (LDH) Assay Kit (Colorimetric) (ab102526) is a quick, convenient and sensitive assay that measures LDH activity in tissue extracts, cell culture supernatant, plasma, serum, urine (UTI), and other biological fluids.

In this colorimetric assay, LDH reduces NAD to NADH, which then interacts with a chromogenic probe to produce a stable, brightly-colored chromophore detectable by absorbance (λ_{max} = 450 nm). The detection range is 1 -100 mU/mL of LDH in samples.

Assay Summary:

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



QUICK ASSAY PROCEDURE

- Preheat plate reader to 37°C
- Solubilize Substrate Mix, LDH Positive Control, NADH Standard I and thaw LDH Assay Buffer
- Prepare samples in duplicate
- Prepare appropriate standard curve
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare and add 50 µL LDH Reaction Mix to each well.
- Read plate in kinetic mode at 37°C for 30 - 60 mins

Precautions & Limitations:

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.
- 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.
- **Interferences:** FBS/BCS or other animal sera contain endogenous LDH and will cause interference if measuring LDH in cell culture medium. This could result in compromised results or complete failure.

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. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date. Aliquot components in working volumes before storing at the recommended temperature. Reconstituted components are stable for 1 month.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
LDH Assay Buffer	50 mL	-20°C	-20°C
Substrate Mix	1 vial	-20°C	-20°C
NADH Standard I	1 vial	-20°C	-20°C
LDH Positive Control	1 vial	-20°C	-20°C

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring absorbance at OD 450 nm
- Double distilled/deionized water (ddH_2O)
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using cells or tissue)
- 1x PBS pH 7.4 (ab285410 or similar)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

NADH Standard I: Reconstitute with 0.4 mL ddH_2O to generate a 1.25 mM NADH Standard Solution. Aliquot and store at - 20°C. Keep on ice while in use. Use within 1 month.

LDH Assay Buffer: Ready to use as supplied. Warm to room temperature before use (a 37°C water bath may help accelerate thawing). Store at -20°C.

LDH Positive Control: Reconstitute the whole vial with 200 µl LDH Assay Buffer. Aliquot and store at - 20°C (avoid repeated freeze/thaw cycles). Keep on ice while in use. Use within 1 month.

Substrate Mix: Reconstitute in 1.1 mL ddH2O by mixing solution for 10 minutes. Store aliquots at -20°C. Use within 1 month.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

1. 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, snap freeze your samples in liquid nitrogen upon extraction and store them 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.

Cell (adherent or suspension) samples:
1. Harvest and count 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 500 µL of cold LDH 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 500 µL of cold LDH 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 500 µL of cold LDH 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.

Note: LDH is unlikely to be detectable in normal urine samples and is only significantly elevated in certain disease or infection states.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

1. Add 0, 2, 4, 6, 8 and 10 µl of reconstituted 1.25 mM NADH Standard I into a series of wells in duplicate in 96 well plate and adjust volume to 50 µl/well with LDH Assay Buffer to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmoles/well of NADH; or prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for 2 replicate curves):

Standard #	Volume of 1.25 mM NADH Standard I (µL)	LDH Assay Buffer (µL)	Final volume standard in well (µL)	End NADH Amount (nmoles/well) Colorimetric Assay
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

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

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).
   Background wells = 50 µL LDH Assay Buffer.
   Positive controls = 2 – 5 µL Positive control (adjust volume to 50 µL/well with LDH Assay Buffer).
2. Each well (standards, samples, and controls) requires 50 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:

X µL component x (Number reactions +1).

Component	Assay Reaction Mix (µL)
LDH Assay Buffer	48
Substrate Mix	2

3. Mix Master Reaction Mix by inversion. Add 50 µL of the Master Reaction Mix to each well. Use a clean tip for each well.
4. Measure output immediately on a microplate reader in kinetic mode at OD every 2-3 minutes at 37°C for at least 30 – 60 minutes.

Note: Incubation time depends on the LDH Activity in the samples. Incubate reaction for up to 4 hours if LDH activity is low. We recommend measuring OD in a kinetic mode, and choosing two time points (T_1 and T_2) in the linear range (OD values A_1 and A_2 respectively) to

calculate the LDH activity of the samples. Standard curve can be read in end point mode (i.e. at the end of incubation time).
Note: If kinetic mode is not available, measure OD 450 nm at T_1 after incubating 37°C for 5 or 10 min to read A_1. This will allow the reaction to stabilize and hit 37°C. Measure again at T_2 after incubating the reaction at 37°C for 30 min (or longer if the LDH activity is low) to read A_2, protect from light. $\Delta A450\ nm = A_2 - A_1$.

Calculations:

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final amount of NADH (nmol).
4. Find the best-fit linear equation of the standard curve using a linear regression and determine the slope.
5. Find the linear range of the sample, and choose two appropriate time points (T_1, T_2) Determine $\Delta A_{450nm} = (A_2 - A_1)$
   Where A_1 is the sample reading at time T_1 and A_2 is the sample reading at time T_2.
   Note: It is essential to read A_1 and A_2 in the reaction linear range, and that the A_1 is taken after the reaction has come to temperature.
6. Use the ΔA_{450nm} to obtain B nmoles of NADH generated by LDH during the reaction time from the standard curve.

$$B = \left(\frac{\Delta A_{450\,nm} - (y - intercept)}{Slope}\right)$$

7. Activity of LDH in the test samples is calculated as:

$$LDH\ Activity = \frac{B}{\Delta T \times V} \times D = {}^{nmol}/_{(min \times mL)} = mU/mL$$

Where:
B = Amount of NADH in sample well calculated from standard curve (in nmoles).
ΔT = Reaction time (in minutes): $\Delta T = T_2 - T_1$
V = Original sample volume added into the reaction well (mL).
D = Sample dilution factor (before adding to well)

Unit Definition = One unit of LDH activity is the amount of enzymatic activity that catalyzes the conversion of lactate to pyruvate, generating 1.0 µmole of NADH per minute at pH 8.8 and 37°C.

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?
Yes, but we typically recommend scraping cells if possible before continuing with the cell sample prep as described above. Trypsinization can be used to detach cells but it is important to be careful since over-trypsinization might damage the plasma membrane and leak LDH into the medium which is removed during sample prep.
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 Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
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