

Lactate Dehydrogenase Activity Colorimetric Assay Kit

(Catalog #K726-500; 500 assays; Store kit at -20°C)

I. Introduction:

Lactate Dehydrogenase (LDH) is an oxidoreductase (EC 1.1.1.27) present in a wide variety of organisms. LDH catalyzes the interconversion of pyruvate and lactate, with the concomitant interconversion of NADH and NAD⁺. When disease or injury damages tissue, cells release LDH into the bloodstream. Being a fairly stable enzyme, LDH activity is widely used to quantify damage. Quantification of LDH thus has a broad range of applications. In this colorimetric assay, LDH reduces NAD to NADH, which then interacts with a probe to produce a color ($\lambda_{max} = 450 \text{ nm}$). The kit quantifies LDH activity in variety of biological samples such as serum or plasma, cells, culture media and fermentation, etc. The assay is quick, convenient, and sensitive. The kit can detect 1 - 100 mU/ml of LDH directly in samples.

II. Kit Contents:

Components	K726-500	Cap Code	Part Number
LDH Assay Buffer	50 ml	NM	K726-500-1
LDH Substrate Mix (lyophilized)	1 Bottle	Amber	K726-500-2
NADH Standard (0.5 μmol , lyophilized)	1 vial	Yellow	K726-500-3
LDH Positive Control (lyophilized)	1 vial	Red	K726-500-4

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm the Assay Buffer to room temperature (RT) before use. Centrifuge all vials briefly prior to opening. All solutions are stable for at least 1 week at 4°C and 1 month at -20°C. Read the entire protocol before the assay.

IV. Reagent Reconstitution and General Consideration:

Substrate Mix: Dissolve with 1.1 ml ddH₂O for 10 min, sufficient for 500 reactions.

NADH Standard Solution: Dissolve NADH Standard into 0.4 ml ddH₂O to generate 1.25 mM NADH Standard solution.

LDH Positive Control: Reconstitute LDH with 200 μl LDH Assay Buffer. Add 2 - 5 μl reconstituted LDH as Positive Control. Keep on ice when using.

1. Sample Preparation:

Homogenize 0.1 g Tissues, or 10⁶ Cells, or 0.2 ml Erythrocytes on ice in 0.5 ml cold LDH Assay Buffer; Centrifuge at 10,000 x g for 15 min at 4°C. Collect the supernatant for assay and store on ice. Serum can be tested directly. Add 2 - 50 μl samples into a 96-well plate. Bring the volume to 50 μl with LDH Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the Standard Curve range.

2. NADH Standard Curve:

Add 0, 2, 4, 6, 8, 10 μl of the 1.25 mM NADH Standard into 96-well plate in duplicate to generate 0, 2.5, 5.0, 7.5, 10.0, 12.5 nmol/well standard. Bring the final volume to 50 μl with LDH Assay Buffer.

3. Reaction Mix:

Mix enough reagents for the number of assays and Standards to be performed. For each well, prepare a total 50 μl Reaction Mix:

- 48 μl LDH Assay Buffer
- 2 μl Substrate Mix Solution

Mix well. Add 50 μl of the Reaction Mix to all samples, Positive Control, and Standards, mix well.

4. Measure OD 450 nm at T₁ to read A₁. Measure again at T₂ after incubating the reaction at 37°C for 30 min (or longer if the LDH activity is low) to read A₂, protect from light. $\Delta A_{450 \text{ nm}} = A_2 - A_1$.

Note: (A) It is essential to read A₁ and A₂ in the reaction linear range. It is more accurate if you observe the reaction progress. Then choose A₁ and A₂ in the linear portion. (B) For Standard Curve, use A₂ reading after 30 min incubation, do not subtract the A₁ reading. The Standard reading is stable for a few hours.

5. Calculation:

Subtract 0 nmol/well NADH background from all readings, plot NADH Standard Curve. Apply the sample $\Delta A_{450 \text{ nm}}$ to the NADH Standard Curve to get B (the NADH amount that was generated between T₁ and T₂).

$$\text{LDH Activity} = \frac{B}{(T_2 - T_1) \times V} \times \text{Sample dilution} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: B is the NADH amount that was generated between T₁ and T₂ (in nmol).

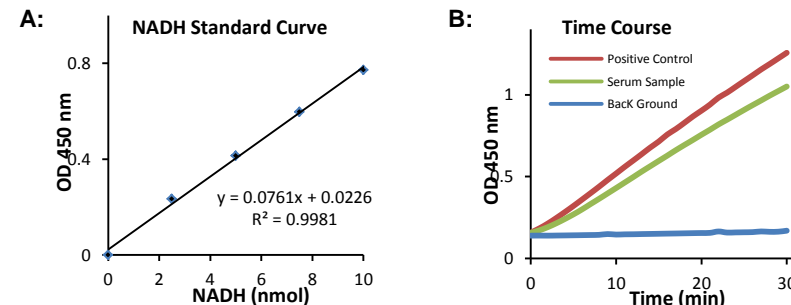
T₁ is the time of first reading (A₁) (in min).

T₂ is the time of second reading (A₂) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

NADH molecular weight: 763.0 g/mol.

Unit definition: One unit of LDH is the amount of enzyme that generates 1.0 μmol NADH per minute at 37°C in our buffer system.



Figures: (A). NADH Standard Curve. (B). Kinetic profiles of approx 0.5 mU of a sample of pure LDH (Positive control) and 2 μl frozen human serum from a commercial source using buffer as a background control.

RELATED PRODUCTS:

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| Glutathione Reductase Assay Kit | Catalase Assay Kit |
| Colorimetric Glutathione Detection Kit | Glucose Assay Kit |
| Glutathione Kit (GSH, GSSG and Total) | GST Fluorometric Assay Kit |
| Phosphate Assay Kit | NAD/NADH Quantification Kit |
| Ammonia Assay Kit | Glutamate Assay Kit |

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.