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 ($\lambda_{\text{max}} = 450$ nm). The kit quantifies LDH activity in various applications 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:

<table>
<thead>
<tr>
<th>Components</th>
<th>K726-500</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH Assay Buffer</td>
<td>50 ml</td>
<td>NM</td>
<td>K726-500-1</td>
</tr>
<tr>
<td>LDH Substrate Mix (lyophilized)</td>
<td>1 vial</td>
<td>Amber</td>
<td>K726-500-2</td>
</tr>
<tr>
<td>NADH Standard (0.5 µmol; lyophilized)</td>
<td>1 vial</td>
<td>Yellow</td>
<td>K726-500-3</td>
</tr>
<tr>
<td>LDH Positive Control (lyophilized)</td>
<td>1 vial</td>
<td>Red</td>
<td>K726-500-4</td>
</tr>
</tbody>
</table>

III. Storage and Handling:
Store kit at ~20°C, protect from light. Warm the Assay Buffer to room temperature 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 Preparations:
Homogenize 0.1 g Tissues, or 10⁶ Cells, or 0.2 ml Erythrocytes on ice in 0.5 ml cold 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 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 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 Assay Buffer
2 µl Substrate Mix Solution
Mix well. Add 50 µl of the Reaction Mix to all samples, Positive Control, and Standard, 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. ∆A₄₅₀ nm = A₂ - A₁.

**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 ∆A₄₅₀nm to the NADH standard curve to get B (the NADH amount that was generated between T₁ and T₂).

$$\text{LDH Activity} = \frac{B}{(T₂–T₁) \times V} \times \text{Sample dilution} = \text{nmol/min/ml} = \text{mU/ml}$$

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.

$$y = 0.0761x + 0.0226$$

$R^2 = 0.9981$

Figure: (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:**
- Glutathione Reductase Assay Kit
- Colorimetric Glutathione Detection Kit
- Glutathione Kit (GSH, GSSG and Total)
- GST Colorimetric Assay Kit
- Acid Phosphatase Assay Kit
- Phosphase Assay Kit
- Pyruvate Assay Kit
- Ammonia Assay Kit
- Catalase Assay Kit
- Glucose Assay Kit
- GST Fluorometric Assay Kit
- Triglyceride Assay Kit
- ADP/ATP Ratio Assay Kit
- NAD/NADH Quantification Kit
- Lactase Assay Kit II
- Glutamate Assay Kit
### GENERAL TROUBLESHOOTING GUIDE:

<table>
<thead>
<tr>
<th>Problems</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>• Use of ice-cold assay buffer</td>
<td>• Assay buffer must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>• Omission of a step in the protocol</td>
<td>• Refer and follow the data sheet precisely</td>
</tr>
<tr>
<td></td>
<td>• Plate read at incorrect wavelength</td>
<td>• Check the wavelength in the data sheet and the filter settings of the instrument</td>
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<tr>
<td></td>
<td>• Use of a different 96-well plate</td>
<td>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</td>
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<tr>
<td>Samples with erratic readings</td>
<td>• Use of an incompatible sample type</td>
<td>• Refer data sheet for details about incompatible samples</td>
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<td></td>
<td>• Samples prepared in a different buffer</td>
<td>• Use the assay buffer provided in the kit or refer data sheet for instructions</td>
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<td></td>
<td>• Cell/ tissue samples were not completely homogenized</td>
<td>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</td>
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<td></td>
<td>• Samples used after multiple free-thaw cycles</td>
<td>• Aliquot and freeze samples if needed to use multiple times</td>
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<tr>
<td></td>
<td>• Presence of interfering substance in the sample</td>
<td>• Troubleshoot if needed</td>
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<tr>
<td></td>
<td>• Use of old or inappropriately stored samples</td>
<td>• Use fresh samples or store at correct temperatures until use</td>
</tr>
<tr>
<td>Lower/ Higher readings in Samples and Standards</td>
<td>• Improperly thawed components</td>
<td>• Thaw all components completely and mix gently before use</td>
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<tr>
<td></td>
<td>• Use of expired kit or improperly stored reagents</td>
<td>• Always check the expiry date and store the components appropriately</td>
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<tr>
<td></td>
<td>• Allowing the reagents to sit for extended times on ice</td>
<td>• Always thaw and prepare fresh reaction mix before use</td>
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<tr>
<td></td>
<td>• Incorrect incubation times or temperatures</td>
<td>• Refer datasheet &amp; verify correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>• Incorrect volumes used</td>
<td>• Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td>Readings do not follow a linear pattern for Standard curve</td>
<td>• Use of partially thawed components</td>
<td>• Thaw and resuspend all components before preparing the reaction mix</td>
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<tr>
<td></td>
<td>• Pipetting errors in the standard</td>
<td>• Avoid pipetting small volumes</td>
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<tr>
<td></td>
<td>• Pipetting errors in the reaction mix</td>
<td>• Prepare a master reaction mix whenever possible</td>
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<tr>
<td></td>
<td>• Air bubbles formed in well</td>
<td>• Pipette gently against the wall of the tubes</td>
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<td></td>
<td>• Standard stock is at an incorrect concentration</td>
<td>• Always refer the dilutions in the data sheet</td>
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<tr>
<td></td>
<td>• Calculation errors</td>
<td>• Recheck calculations after referring the data sheet</td>
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<td>• Substituting reagents from older kits/ lots</td>
<td>• Use fresh components from the same kit</td>
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<tr>
<td>Unanticipated results</td>
<td>• Measured at incorrect wavelength</td>
<td>• Check the equipment and the filter setting</td>
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<tr>
<td></td>
<td>• Samples contain interfering substances</td>
<td>• Troubleshoot if it interferes with the kit</td>
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<tr>
<td></td>
<td>• Use of incompatible sample type</td>
<td>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</td>
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<td></td>
<td>• Sample readings above/below the linear range</td>
<td>• Concentrate/ Dilute sample so as to be in the linear range</td>
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**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.