# SIGMA-ALDRICH®

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# Product Information

**Lactate Dehydrogenase Activity Assay Kit**

Catalog Number **MAK066**
Storage Temperature –20 °C

# TECHNICAL BULLETIN

## Product Description
Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyses the interconversion of pyruvate and lactate. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissue and cells. LDH is also elevated in certain pathological conditions such as cancer. Quantification of LDH has a broad range of applications.

The LDH Activity Assay kit quantifies LDH activity in variety of biological samples. The assay is quick, convenient, and sensitive. In this kit, LDH reduces NAD to NADH, which is specifically detected by colorimetric (450 nm) assay.

## Components
The kit is sufficient for 500 assays in 96 well plates.

| | |
|---|---|
| LDH Assay Buffer     Catalog Number MAK066A | 50 mL |
| LDH Substrate Mix     Catalog Number MAK066B | 1 vl |
| NADH Standard, 0.5 µmole     Catalog Number MAK066C | 1 vl |
| LDH Positive Control, 20 µL     Catalog Number MAK066D | 1 vl |

## Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.

## Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

LDH Assay Buffer – Allow buffer to come to room temperature before use.

LDH Substrate Mix – Reconstitute in 1 mL of water. Mix well by pipetting and keep cold while in use. Substrate Mix is stable for one week at 4 °C and 1 month at –20 °C.

1.25 mM NADH Standard – Reconstitute in 400 µL of water to generate 1.25 mM standard solution. Mix well by pipetting and keep cold while in use. The NADH standard solution is stable for one week at 4 °C and 1 month at –20 °C.

LDH Positive Control – Dilute the LDH Positive Control 10-fold (1:9) with LDH Assay Buffer before use and use 2–5 µL of the diluted LDH Control as positive control.

## Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

## Procedure
All samples and standards should be run in duplicate.

### NADH Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 $\mu$L of the 1.25 mM NADH Standard in duplicate into a 96 well plate, generating 0 (blank), 2.5, 5, 7.5, 10, and 12.5 nmole/well standards. Add LDH Assay Buffer to a final volume of 50 $\mu$L.

### Sample Preparation
Tissue (100 mg), cells ($1 \times 10^6$), or erythrocyte (200 $\mu$L) samples should be rapidly homogenized on ice in 500 $\mu$L of cold LDH Assay buffer. Centrifuge at 10,000 $\times$ $g$ for 15 minutes at 4 °C to remove insoluble material. Use soluble fraction for assay. Serum samples may be assayed directly. Add 2–50 $\mu$L samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 $\mu$L with LDH Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

### Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 $\mu$L of the Master Reaction Mix is required for each reaction (well).

**Table 1.**
Master Reaction Mix

| Reagent | Master Reaction Mix |
|---|---|
| LDH Assay Buffer | 48 $\mu$L |
| LDH Substrate Mix | 2 $\mu$L |

2. Add 50 $\mu$L of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation.

3. After 2–3 minutes, take the initial measurement ($T_{initial}$). Measure the absorbance at 450 nm at the initial time ($A_{450}$)$_{initial}$.
Note: It is essential ($A_{450}$)$_{initial}$ is in the linear range of the standard curve.

4. Incubate the plate at 37 °C taking measurements ($A_{450}$) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement [($A_{450}$)$_{final}$] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is $T_{final}$.
Note: It is essential the final measurement falls within the linear range of the standard curve.

**Results**

Calculations

Correct for the background by subtracting the final measurement $[(A_{450})_{final}]$ obtained for the 0 (blank) NADH standard from the final measurement $[(A_{450})_{final}]$ of the standards. Background values can be significant and must be subtracted from all readings. Plot the NADH standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to $T_{final}$ for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Compare the $\Delta A_{450}$ of each sample to the standard curve to determine the amount of NADH generated by the kinase assay between $T_{initial}$ and $T_{final}$ (B).

The LDH activity of a sample may be determined by the following equation:

$$\text{LDH Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between $T_{initial}$ and $T_{final}$.

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

LDH activity is reported as nmole/min/mL = milliunit/mL One unit of LDH activity is defined as the amount of enzyme that catalyzes the conversion of lactate into pyruvate to generate 1.0 μmole of NADH per minute at 37 °C.

Example:

NADH amount (B) = 5.84 nmole

First reading ($T_{initial}$) = 3 minute

Second reading ($T_{final}$) = 32 minutes

Sample volume (V) = 0.01 mL

Sample dilution is 1

LDH activity is:

$$\frac{5.84 \times 1}{(32-3) \times 0.01} = 20.14 \text{ milliunits/mL}$$

**Troubleshooting Guide**

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay not working | Ice Cold Assay Buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | For colorimetric assays, use clear plates |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if samples will be used multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Master Reaction Mix before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the reaction mix |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare a Master Reaction Mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the tubes |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |

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