SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

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.

Component	Amount
LDH Assay Buffer Catalog Number MAK066A	50 mL
LDH Substrate Mix Catalog Number MAK066B	1 vial
NADH Standard, 0.5 μmole Catalog Number MAK066C	1 vial
LDH Positive Control Catalog Number MAK066D	1 vial

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 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 – Reconstitute in 200 μL of LDH Assay Buffer before use. Use 2–5 μL of the prepared LDH Control as positive control. Keep on ice when using.

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 μL 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 μL.

Sample Preparation
Tissue (100 mg), cells (1 × 10^6), or erythrocyte (200 μL) samples should be rapidly homogenized on ice in 500 μL of cold LDH Assay buffer. Centrifuge at 10,000 × *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 μL samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 μL 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 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Master Reaction Mix
LDH Assay Buffer	48 μL
LDH Substrate Mix	2 μL

2. Add 50 μL 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 ΔA_{450} of each sample to the standard curve to determine the amount of NADH generated by the assay between $T_{initial}$ and T_{final} (B).

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

LDH Activity =

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

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:

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

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

KNV,KVG,SG,LS,VC,MF,MAM 03/18-1

©2018 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.